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BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ

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PHYSIOLOGY

CORRELATION BETWEEN CHANGES IN HIGHER NERVOUS ACTIVITY AND THE SECRETORY FUNCTION OF THE STOMACH DURING B_1-AVITAMINOSIS IN DOGS

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A description was given in a previous communication [5] of the main consistent changes in gastric secretion in dogs during the development of B_1-avitaminosis and during the subsequent period of vitamin therapy. We showed that B_1-avitaminosis caused marked disturbances of the secretory activity of the gastric glands. It was also established that systematic vitamin B_1 therapy diminished the clinical manifestations of the avitaminosis and led to the return of the impaired gastric secretion to a nearly normal level.

Our further investigations showed that repeated vitamin B_1 deficiencies were associated with still more marked disturbances of gastric gland function with diminution of secretion. In the profound stages of this condition there is almost complete depression of gastric secretion, which is only slowly restored, despite vitamin therapy [4].

Comparison of the results of physiologic investigations with the histologic data showing absence of organic lesions of the glandular cells of gastric mucosa suggested that the impairment of secretory activity of the gastric glands in vitamin B_1 deficiency was of functional character and resulted from impairment of nervous regulatory mechanisms.

It is known from the literature, and has been demonstrated by our studies, that a vitamin B_1 deficit causes considerable disturbances in bodily functions. It is also known [3] that vitamin B_1 plays a most important role in metabolism, in particular the metabolism of various tissues, such as nerve, gland etc. Metabolic disturbances in glandular tissue may be implicated in the development of changes in the gastric secretory function observed by us during vitamin B_1 deficiency. It could be supposed that vitamin B_1 deficiency in the organism elicited gastric secretory dysfunction by this means and also by way of changes in the central nervous system and its highest level, viz.,the cerebral cortex. L.O. Zeval'd studied the effect of vitamin B_1 deficiency on the conditioned reflex activity in dogs and found that as the result of such deficiency there were marked disturbances of conditioned reflex activity which continued for a long time, even after termination of the vitamin deficiency [1].

Following the determination of the main consistent changes in gastric secretion during vitamin B_1 deficiency we attempted to analyze the mechanism of the observed disturbances associated with this pathologic process. Consequently,it was necessary first of all to discover the correlation between disturbances of higher nervous activity and the secretory function of the stomach in the same experimental dogs during the development of vitamin B_1 deficiency.

EXPERIMENTAL METHOD

Experiments were performed on dogs with isolated gastric pouches (Brestkin-Savich) and parotid gland fistulas.

* Deceased.

The effect of B_1-avitaminosis on gastric secretion and on higher nervous activity was studied. Experiments concerned with gastric secretion and with conditioned reflex activity were staged alternately, every other day. A background was first established; subsequently the clinical picture of developing avitaminosis was recorded systematically.

Induction of experimental vitamin B_1 deficiency in dogs has been described in detail previously [2]. In the present work, results obtained on the dogs Zhuk and Mal'chik are described; the vitamin deficiency was induced in these animals repeatedly (for the third and fourth time) by maintaining them on a saccharose-casein diet, free from vitamin B_1, as described in previous experiments.

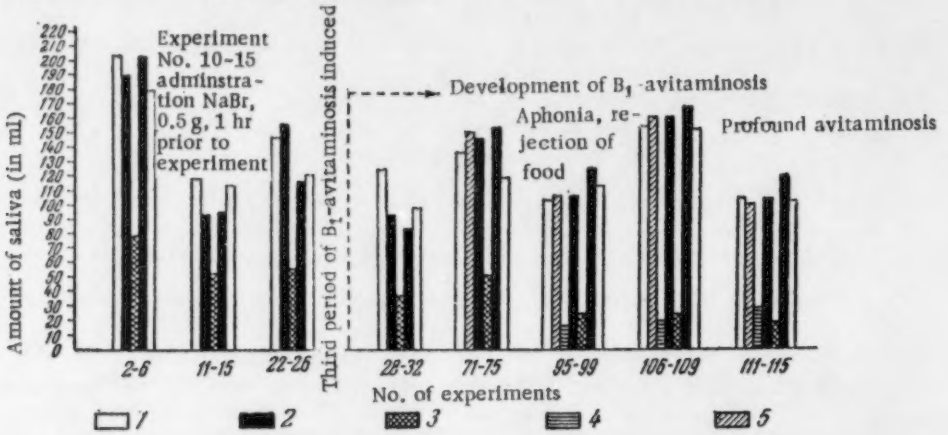

Fig. 1. Higher nervous activity of the dog Zhuk during the recovery period following the second period of B_1-avitaminosis and during the development of the third period of B_1-avitaminosis (arithmetic mean values of conditioned reflexes in 5 experiments).

1) Light; 2) M_{120}; 3) M_{60}; 4) buzzer; 5) bell.

In our first communication we described the experimental procedure for studying gastric secretion. The method of formation and investigation of conditioned reflexes was the usual one practised in I. P. Pavlov laboratories. Investigations of the higher nervous activity of the dogs Zhuk and Mal'chik were begun during the period of recovery, after vitamin therapy when the clinical manifestations of avitaminosis had disappeared. Conditioned stimuli included light, metronome at the rate of 120 strokes per minute (M_{120}) and metronome at the rate of 60 strokes per minute (M_{60}, differentiation). Then during the development of avitaminosis two new conditioned stimuli were introduced: a bell and a differentiation stimulus, viz., a buzzer. The conditioned stimuli were used in a stereotyped order (Fig. 1). The amount of saliva secreted was recorded on the scale.

When a more or less stable system of positive and negative reflexes had been established in the experimental dogs they were again put on the avitaminosis regimen – saccharose-casein diet.

EXPERIMENTAL RESULTS

Experimental data obtained on the dog Zhuk (Figs. 1 and 2) will serve to illustrate the main results of investigation of higher nervous activity and gastric secretion during vitamin B_1 deficiency.

Fig. 1 shows the arithmetic mean values of the conditioned reflexes obtained in 5 experiments during various periods of the investigation in Zhuk, extending over 1 year and 2 days (January 29, 1952 to January 31, 1953). The mean values of experiments characteristic for the given period of illness are given. Fig. 2 presents the mean arithmetic values of Zhuk's gastric secretion during the same periods.

Zhuk, who had sustained B_1-avitaminosis twice and had been in a recovery period for 3 months and 8 days, and was apparently quite healthy, showed impairment of strength relationships during the action of positive conditioned stimuli (light and M_{120}), and incomplete differentiation to M_{60} (experiments Nos. 2-6). Complete differentiation could not be established after a 10-day period of sodium bromide administration to the dog (0.5 g in milk one hour prior to experiment), when the value of the positive reflexes diminished. When the value of conditioned re-

flexes approached relatively normal levels (experiments No. 22-26) the dog was put on the saccharose-casein diet for the third time.

From the very first days of the avitaminosis regimen the dog showed lower values of conditioned reflexes, paradoxic relations between the values of conditioned reflexes to light and M_{120} (experiments No. 28-32), and lowering of gastric secretion from 7.1-4.8 to 2.4 ml in response to meat, and from 5-8.5 to 3.7-1.9 ml in response to alcohol (Fig. 2).

The dog's behavior was unstable during the subsequent period of avitaminosis development: marked excitation alternated with sluggishness, increased appetite with refusal to take food. During this stage of the illness considerable fluctuations in the amount of gastric juice were noted, as well as changes in the latent period of secretion, acidity and digestive ability of the juice in response to both stimuli (Fig. 2) and substantial fluctuations in the values of the conditioned reflexes (Fig. 1).

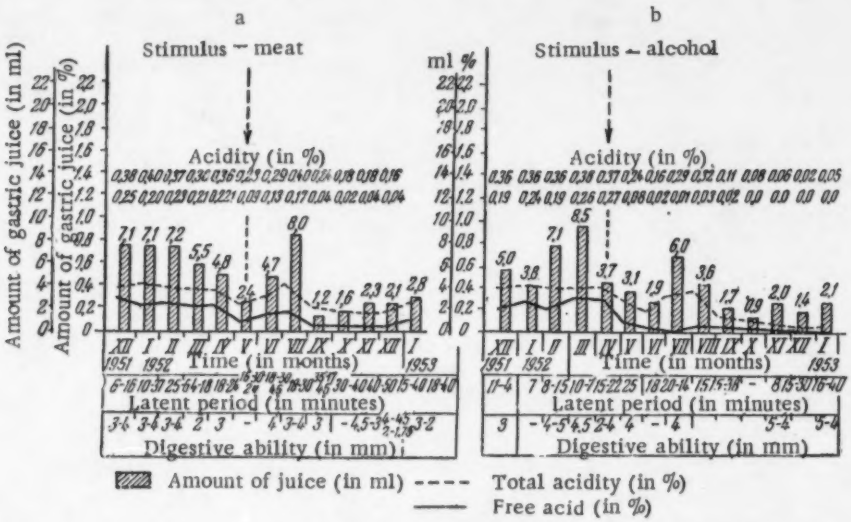

Fig. 2. Gastric secretion in the dog Zhuk during the recovery period following repeated B_1-avitaminosis and during the development of the third period of B_1-avitaminosis. a) Gastric secretion in response to 100 g autoclaved meat (vertical broken line divides the recovery period after the second bout of B_1-avitaminosis from the B_1-avitaminosis induced for the third time); amount of juice — arithmetic means of experiments staged during the months; acidity — arithmetic mean of the same experiments; latent period and digestive ability — also for the month with fluctuations; b) gastric secretion in response to 100 ml 10% alcohol. (Designations the same as in Fig. 2, a).

During the 5th month of the development of avitaminosis a new conditioned stimulus, a bell, was introduced into the previously elaborated system. In the first 5 experiments marked fluctuations in the magnitude of the conditioned reflexes were noted, but in the 6th experiment the reflex to the bell had already become more or less stable in value. During the 6th month of the illness, when profound B_1-avitaminosis had developed and the magnitude of all the conditioned reflexes had diminished, a differentiation stimulus with respect to the bell, viz., a buzzer, was introduced into the system of conditioned stimuli. Records of 2 experiments are presented in the table.

Changes in higher nervous activity and secretory function of the stomach during repeated B_1-avitaminosis have been considered in the case of one of our experimental dogs. Basically similar data, but with even greater depression of gastric secretion, were obtained in the experiments on the dog Mal'chik.

Analysis of the experimental results indicates that repeated avitaminosis evokes definite changes in cerebral cortical function. These changes consist mainly of impaired relationship between excitation and inhibition. During the stage of development of profound avitaminosis weakening of both excitation and inhibition is noted from the marked drop in the values of positive conditioned reflexes, disinhibition of differentiations and phasic states.

The experiments also showed that during repeated B_1-avitaminosis new conditioned reflexes (bell and buzzer) could be formed by dogs even during profound vitamin B_1 deficiency.

Experimental Data Obtained on the Dog Zhuk

Interval in seconds	Number of combinations	Conditioned stimulus	Latent period in seconds	Amount of secretion		Remarks
				Conditioned	Unconditioned	
			Experiment No. 96, 12/2/1952			
5	296	+ Light	2	165	200	Zhuk during the development of third period of avitaminosis. Dog very ill
5	33	+ Bell	2	145	200	
5	17	— Buzzer	10	30	25	
5	191	+ M_{120}	4	120	300	
5	95	— M_{60}	28	5	10	
5	192	+ M_{120}	3	125	300	
5	297	+ Light	3	130	280	
			Experiment No. 97, 12/4/1952			
5	298	Light	3	70	280	Dog very ill
5	34	Bell	3	30	275	
5	18	Buzzer	20	10	5	
5	193	M_{120}	5	70	300	
5	96	M_{60}	30	0	10	
5	194	M_{120}	6	65	290	
5	299	Light	3	50	270	

Repeatedly induced B_1-avitaminosis leads to marked disturbances of gastric secretory function in dogs. During the development of avitaminosis sharp fluctuations in gastric secretion (hypersecretion and hyposecretion) are observed, as well as changes in the qualitative composition of the gastric juice. During the stage of profound avitaminosis secretion is inhibited. Comparison of changes in higher nervous activity with changes in gastric secretion during the development of B_1-avitaminosis shows that their onset is, as a rule, simultaneous.

During the recovery period the conditioned reflex activity of the salivary glands returns to normal more rapidly than the secretory function of the gastric glands.

The aim of our further investigations is to discover which disturbances of nervous regulation lead to disorders of gastric secretory function with a given pathologic process.

SUMMARY

The author studied the effect of B_1-avitaminosis on the higher nervous activity and the secretory function of the stomach in the same experimental animals. Experiments performed on dogs with an isolated stomach pouch and fistula of the parotid gland demonstrated that B_1-avitaminosis, induced repeatedly, brought about significant disturbances of the higher nervous activity, manifested by decrease of the value of positive conditioned reflexes, disinhibition of differentiations in the phasic conditions, as well as by significant disturbances of the secretory function of the gastric glands. The study of changes occuring in higher nervous activity and changes of gastric secretion, during the development of B_1-avitaminosis demonstrated that they appeared, as a rule, simultaneously.

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* In Russian.

** Original Russian pagination. See C. B. translation.

TONIC REFLEXES IN MAN

COMMUNICATION III. TONIC CERVICAL REFLEXES IN HEALTHY ADULTS AND THE INFLUENCE OF UNSTABLE POSITION ON THESE REFLEXES

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Among tonic reflexes an important part is played by reflexes from cervical muscle (or vertebro-cranial articulation [10]) proprioceptors when the position of the head is altered with respect to that of the trunk. R. Magnus [9] established that these reflexes were also important in the system of static and of static-kinetic reflexes and also demonstrated their close interrelation with labyrinthine and proprioceptive trunk reflexes. I. S. Beritov [1] concluded that the impulses from the cervical and labyrinthine receptors were in themselves too weak to elicit a reflex but enhanced the excitability of centers which coordinate these reflexes,so that activity occurred whenever some additional stimulus was brought into play. There have been many attempts at detecting these reflexes in man. They were observed in clinical practise by M. Minkowski [11], H. Stenvers [13], P. Simons [12]. Krol', Markov and Kantor [7] observed diminution of chronaxie in the forearm extensors on the "chin" side when the head was turned to the side and interpreted this as indication of increased tone; their subjects were healthy adults. A. N. Krestovnikov [5] also observed healthy adults, using the roentgenokymograph method, and obtained increased diaphragm tone on the "chin" side and decreased tone on the opposite side.

Our aim was to investigate the changes in tone on altering the position of the head in healthy people (cervical tonic reflexes) and the effect of unstable body posture on these reflexes.

EXPERIMENTAL METHOD

Experiments were performed on healthy adults (students of the Institute of Physical Culture) who took part in sports but were not training during recuperation from some somatic disorders. The degree of muscle tone was measured by the O. V. Plotnikova and I. I. Shaffer [8] tonometer. Changes in muscle tension were recorded graphically by myotonography [3, 4] from both deltoid muscles; these records were taken in the sitting position with hands folded in lap and in the "standing at ease" position. Myographic records were also taken of the "voluntary" tension of both pectoralis major muscles on changing the position of the head at a command from the experimenter and the involuntary reflex change in tone in both deltoid muscles.

EXPERIMENTAL RESULTS

Cervical tonic reflexes are detected myotonographically as slight but definite increases in the tension of the deltoid muscle contralateral to the side toward which the head is inclined. In some cases diminished muscle tone was noted in the ipsilateral deltoid at the time when the tone was increased in the deltoid contralateral to the direction in which the head was inclined. Inclination of the head backward increased the tone of both muscles, while inclination forward most frequently decreased the tone of both muscles, but sometimes increased it somewhat or produced no change. Turning the head to the side increased the muscle tone bilaterally but more so on

the ipsilateral side (Fig. 1). The myotonogram shows clearly the difference between the high, steeply rising and falling "voluntary" tension of the pectoralis major muscles and the low, slowly rising tonic reflex tension of the deltoid muscles.

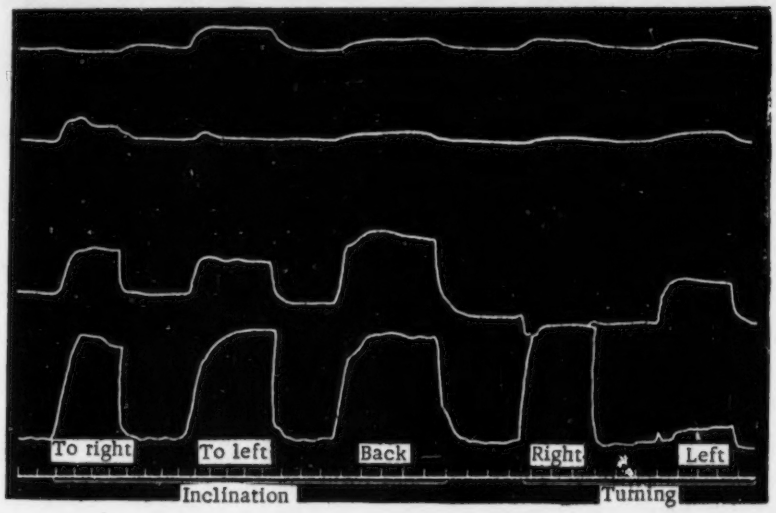

Fig. 1. Myotonograms of both pectoralis major muscles and deltoid muscles in subject N.K., male, 23 years old. Standing "at ease" position; various changes in the position of the head (in the following order: inclination to the right, left, back, turning to the right, left).
Records from above down: right deltoid, left deltoid, right pectoralis major, left pectoralis major muscle; time marker (1 second).

Tonometric data were in complete accord with this. The greatest difference compared to the initial position of the head was noted when the head was inclined sideways and backward reaching +10 - 12 mm Hg in adults, corresponding to 33% of the initial background.

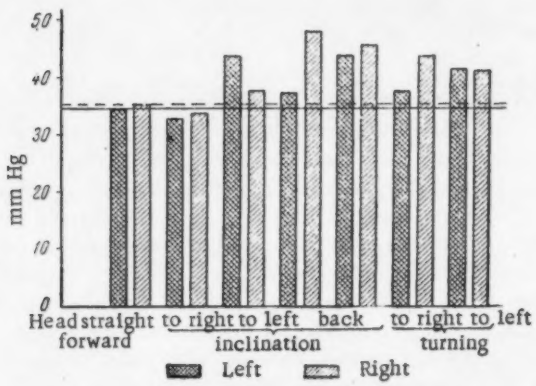

Fig. 2. Diagram of tonometric data for both deltoid muscles in subject A.M., 22-year-old male, during different positions of the head.
Ordinate: values of muscle tension in millimeters of mercury. Initial level (with the head held straight) denoted by a solid line (for the left deltoid muscle) and a broken line (for the right deltoid muscle).

The diagram presented in Fig. 2 can serve as an illustration. It must be noted that these reflexes are more marked in subjects who are not undergoing systematic physical training. Those who are undergoing such training show much smaller reflexes and only when the head is inclined sideways. Such positions are almost nonexistent in exercises and we suppose that cervical tonic reflexes are preserved in such subjects for the very reason that they are unusual. It appears that in all the other positions which are common in athletics the conditioned reflex inhibition suppresses the cervical tonic reflexes.

The main significance of tonic reflexes lies in their contribution to preservation of balance. Body balance is very unstable, viewed from the standpoint of mechanics, but human adults maintain their balance very well physiologically in a great variety of positions. This must evidently be ascribed to perfection of the

mechanism of unconditioned and conditioned tonic reflexes. The course followed by tonic reflexes under various conditions of equilibrium is thus of great interest.*

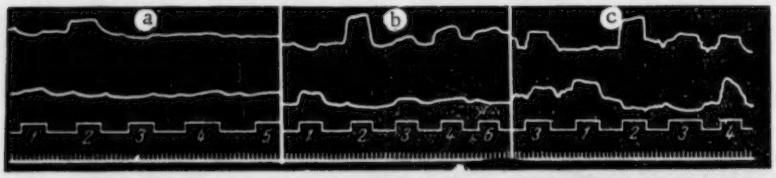

Fig. 3. Myotonograms of the deltoid muscles: a) in the sitting position; b) in the standing position; c) standing on a springy rubber membrane. Records from above down: right deltoid muscle, left deltoid muscle, marker showing changes in position of the head: 1) inclination to the right; 2) inclination to the left; 3) inclination backward; 4) turning to the right; 5) turning to the left; time marker (1 second).

We therefore repeated the studies on cervical tonic reflexes in a single experiment including some of the 5 different positions of the body: I – sitting, II – standing "at ease," III – standing "at ease" with eyes closed, IV – standing on a springy rubber membrane, V – standing on a springy rubber membrane with eyes closed (this position was only used in the case of subjects with good balance preservation). Objective recording in these experiments was only monitored by myotonographic records [5, 8] from the two deltoid muscles.

When the subject changed from one position to another with progressively more difficult conditions for maintaining his balance there was growing increase of cervical tonic reflexes. A typical case was that of subject A. M. (male, 22 years old). Fig. 3, a shows the myotonogram recorded in position I (sitting, hands folded in lap). The tonic reflexes are small; well-defined increase of tone was only noted on the contralateral side when the head is inclined sideways. Reflexes increase markedly when the subject is made to assume position II (Fig. 3, b). The most marked change is seen in the right deltoid muscle. Inclinations sideways are accompanied by simultaneous increase of tone contralaterally and definite decrease of tone ipsilaterally; inclination of the head backward is associated with a small bilateral increase of tone. Position III causes an increase in tone in many subjects but evokes tremor more frequently and sometimes differs little from position II. Very characteristic are the phenomena arising in position IV (Fig. 3, c). It is associated with very pronounced tremor, occasionally with loss of balance; the subject raises his arms, makes a step and even jumps off the rubber. The reflexes are pronounced. Fig. 3, c) draws attention not only to the marked increase of reflexes and tremor but also to the generally unstable level of tonic tension as reflected in the fluctuations of the traces for the right and left deltoid muscles which had been sufficiently stable as can be seen from Fig. 3, a.

The influence of unstable position on the cervical tonic reflexes varies, depending on the physical training of the subjects. The picture described above applies to persons who are not engaged in sports or athletics. Those who are so engaged, and especially athletes and acrobats, are continuously exercising their ability to maintain body balance in very complex positions and therefore exhibit less marked changes in reflexes on changing the position of the body. An additional position V – standing on the rubber with eyes closed – was used for trained athletes. This position is sustained with difficulty even by first-rate athletes, causing loss of balance and performance of balancing movements. It is particularly difficult to maintain balance when inclining the head backwards; this causes not only tonic but also phasic contractions of the muscles and gross movements of the whole body. Consequently, the cervical tonic reflexes become more apparent as the conditions become more unfavorable for preservation of balance; the reason for this is evidently that in a new and particularly in an unaccustomed position of the body (on the rubber sheet) the unconditioned reflex regulation of tone resumes an important role, and the cervical tonic reflexes are a component of this regulation. When trained athletes and acrobats are subjected to the most complex conditions and the tonic reflexes become weaker – always accompanied by increased general tension of muscles – it evidently indicates that in such cases the previous formation of appropriate natural conditioned reflexes transfers the function of balance preservation to the cerebral cortex which sends increased impulses to the body

* The influence of unstable position on the proprioceptive reflex described by A. A. Ukhtomskii has already been investigated in our laboratory by A. S. Dmitriev [2].

musculature and balance is maintained by diffuse tension of the muscles.

This fact confirms the earlier findings of A. N. Krestovnikov [6] that the role of proprioceptive sensation increased when the body found itself without support or inadequately supported. It follows that both in life, and under conditions of physiologic experiment, increasing difficulty in maintaining equilibrium leads to increased importance of the cervical tonic reflexes. As regards the mechanism of this phenomenon, it may be supposed that the direct cause responsible for the lowering of the cervical tonic reflex threshold, upon impairment of balance, is excitation of the vestibular analyzor receptors.

SUMMARY

The cervical tonic reflexes were investigated on deltoid muscles of healthy persons. Myotonographic and tonometric methods were used. It was revealed that when the head was bent backward , the tone increased on both sides, when the head was bent to one side the tone increased on the opposite side, whereas when the head was turned, the tone increased on both sides with the prevalence on the side to which it was turned. The reflexes were more pronounced in untrained persons and less so in trained athletes. The reflexes were considerably increased when the body was in an unstable position.

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THE MOTOR FUNCTION OF THE INTESTINE WHEN THE STOMACH IS EMPTY

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The first attempt to demonstrate various types of digestive tract activity and to relate their origin to the influence of prolonged dietary regimens was made in I. P. Pavlov's laboratories [11].

V. N. Boldyrev [2] was the first to discover that, when not engaged in digestion, the periodic activity of the stomach could be lastingly replaced by continuous activity. I. A. Edel'man [12] distinguished two types of movement of the empty stomach: periodic and "acidic." The final concept of the types of motor activity of the fasting stomach was given by S. V. Anichkov [1] who described periodic contractions (type A), strong continuous "acidic" contractions (type B), and weak continuous contractions with increased gastric tone (type C).

It was later shown [1, 7, 13] that type A was characteristic for adult dogs, while continuous "acidic" contractions of the stomach were observed predominantly in pups and young monkeys.

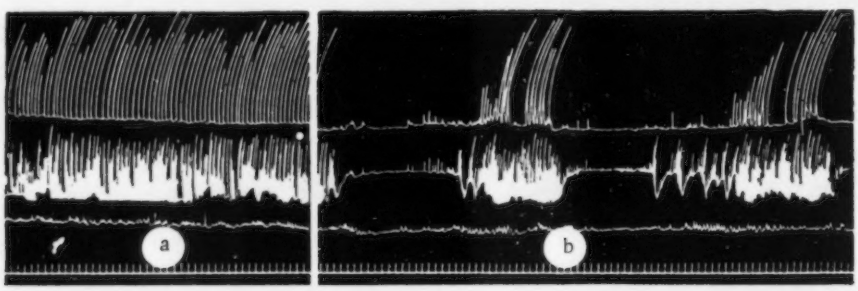

Fig. 1. Continuous and periodic motor activity of the digestive tract when not engaged in digestion (dog Mal'chik).
a) Continuous activity; third type of motor function of the duodenum; b) periodic activity; first type of motor function of the duodenum.
Records from above down: gastric motor activity; duodenal motor activity; sigmoid motor activity; time marker (3 minutes 20 seconds).

Various types of periodic and continuous intestinal activity, in particular duodenal, have also been described [4, 10, 3, 6, 8].

A number of authors explain the absence of periodic motor activity of the duodenum by the mechanical effect of the balloon introduced into its lumen. At the same time periodic motor activity can be clearly observed by oncographic means on an intestinal loop externalized under the skin [5]. Since it is reasonable to assume that the various types of motor activity pertaining to the stomach when not engaged in digestion must to some extent reflect the state of the whole digestive system, we supposed that the upper portion of the small intestine would probably also reflect various functional states of its motor activity. The present work was undertaken to test this hypothesis.

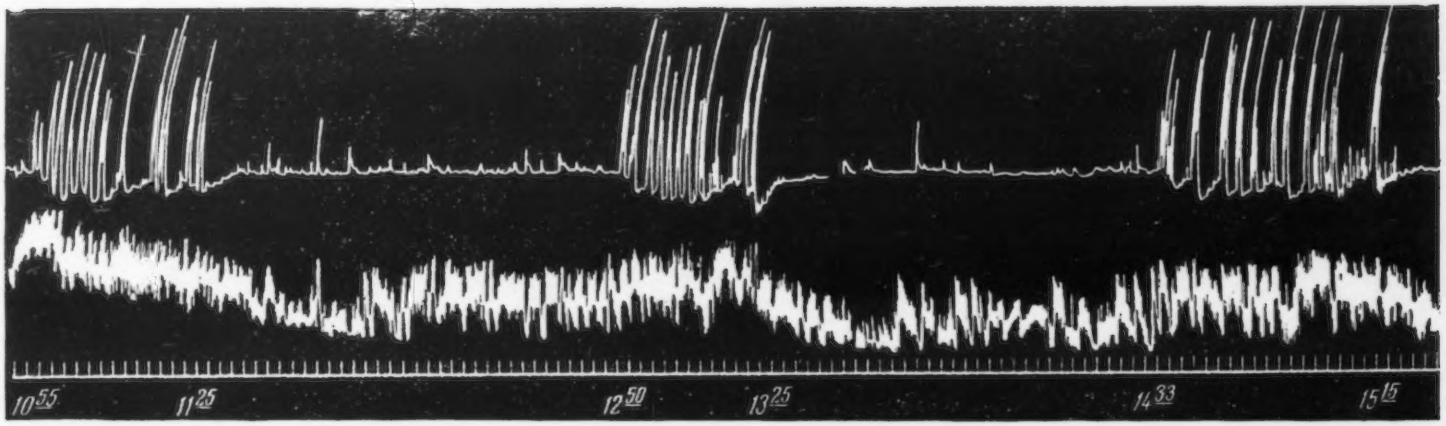

Fig. 2. Periodic motor activity of the stomach and intestine. Second type of duodenal motor activity (dog Butuz). Records from above down: gastric motor activity; duodenal motor activity; time marker (3 minutes 20 seconds).

EXPERIMENTAL METHOD

Experiments were carried out on four dogs with gastric and duodenal fistulas. The experiments were performed 18-20 hours after a meal. In order to check the effect of qualitatively different diet on the motor function of the intestine, the dogs were sometimes kept on a predominantly milk diet, sometimes on a meat diet. The duration of the experiments was from 5 to 24 hours. The intestinal movements were recorded by the Boldyrev method on a kymograph with a slowly rotating drum. The volume of the balloon used for recording the duodenal contractions was in all cases the same (8-10 cm^3). The motor activity of the stomach and the duodenum was recorded simultaneously. A total of 55 experiments was performed, 15 of these covering a period of 24 hours each.

EXPERIMENTAL RESULTS

When the stomach and the upper part of the small intestine were free of food, three main types of motor activity could be noted in the duodenum and the upper portion of the intestine.

The first type, is definite periodic acitivity, and is characterized by an almost complete absence of duodenal contraction during the "resting period". The periods of contraction are considerably shorter than the corresponding resting periods of the stomach and continue for an average of 30-40 minutes. 10-30 minutes prior to the beginning of gastric activity fluctuations in intestinal tone and intestinal contractions usually appear. The frequency of intestinal contractions is 14-18 per minute. The wave-like movements occur in 1-3 minute periods, at first infrequent, then merging together. They constitute the period of duodenal "activity"; its duration is 50-80 minutes. As a rule, the intestinal contractions end 5-10 minutes later than the gastric activity. The intestinal tone is somewhat raised during the contractions, but in a number of experiments diminution of tone was noted (Fig. 1, b). It is this particular type of periodic motor activity of the duodenum which has been described in the literature [9, 10]. We observed it only in one of the four experimental dogs. It is accompanied by strictly periodic secretions by the pancreas and intestine and output of bile during the motor activity of the duodenum [9].

The second type of periodic motor activity of the duodenum, which may be called intermediate, is expressed mainly in prolonged changes of tone.

Throughout the experiment, the duodenum contracts continuously at a rate of 12-16 contractions per minute. The contractions are grouped together (in lots of 15-30) into waves with a periodicity of 1-3 minutes, which follow one another continuously. During each such wave there is first an increase in tone and some enhancement of contraction, then diminution of tone and weakening of contractions. Gradual increase of intestinal tone and marked enhancement of contraction correspond to each period of gastric activity. However, in some experiments, no change in the strength of intestinal contraction was noted. The lowest intestinal tone and the weakest contractions are observed 10-30 minutes after termination of gastric activity. On the contrary, when the stomach is undergoing periodic contractions the intestinal tone is at its highest. It must be stressed that despite the continuous character of contraction of this type, the secretion of pancreatic juice, intestinal juice and bile occur strictly periodically. Secretion of the juice by the duodenum begins before the start of periodic gastric contractions and ends a few minutes after the latter's termination. Evidently there is an interconnection between the periodic secretion of duodenal juice, periodic gastric contractions and periodic increases in duodenal tone, which is demonstrable in the presence of continuous movements of the duodenum. The second type of duodenal contraction corresponds to that described earlier [3]. We observed it in three of our experimental dogs (Fig. 2).

The third type of duodenal motor activity is expressed by continuous contractions of great force over a period of 6-10 hours without consistent fluctuations in tone. The rhythm of these contractions is slower (4-8 per minute) than that observed during periodic activity of the duodenum (Fig. 1, a). The duodenal contractions are accompanied by continuous contractions of the stomach, copious secretion of gastric juice, and very weak but prolonged secretion by the duodenum.

The first and second types of duodenal motor activity are evidently a consequence of individual peculiarities of different animals, since over a period of many months we hardly observed replacement of one type by the other. The duration of the experiments was without significance in this respect; nor did prolonged uniform diet or change of dietary regimen lead to a change in the type of activity.

The third type of activity was observed in all the four dogs and replaced the first or second types. Evidently, it depends not on the individual peculiarities of the animal, but on the functional state of the digestive tract. This is supported by the presence of transition from periodic duodenal contractions to continuous ones in the course of

the same experiment. Such transitions could be observed only in experiments lasting 24 hours; there was no strict correlation with the time of day, although continuous contractions were more often observed during the night and in the morning.

It seems to us that our material indicates caution with regard to erroneous conclusions concerning the existence of only periodic or continuous motor activity of the duodenum. The recording method used by us and other authors permits the demonstration of both types of duodenal motor activity. The first and third types of activity are clearly defined, but the second type is more blurred. We refer it to periodic activity only provisionally.

In favor of delineating this type as a special form of periodic duodenal activity is the presence of accompanying manifestations which are associated with continuous motor activity of the duodenum: continuous gastric contractions, continuous secretion of acid gastric juice, weak and continuous secretion by the duodenum.

Our experiments thus confirm the close functional interconnection which exists between gastric and duodenal activity when not engaged in digestion.

SUMMARY

Three types of motor function of duodenum were revealed in chronic experiments on 4 dogs with gastric and duodenal fistulas. The first two types pertain to periodic function, while the third is a continuous intestinal motor function. In prolonged experiments the periodic duodenal motor function of the first and second type is changed into continuous contraction of the third type, which demonstrates the changes in the functional condition of the digestive tract. The first two types of motor function reflect the individual peculiarities of the digestive tract of various animals since one is not changed into another. An interrelationship was established between different forms of gastric and duodenal motor functions.

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THE EFFECT OF FOOD STIMULI ON LEUKOCYTOSIS IN MAN

COMMUNICATION I. THE SIGNIFICANCE OF VARIOUS FOODS IN THE CHARACTERISTICS OF CONDITIONED AND COMPLEX REFLEX FOOD-INDUCED LEUKOCYTOSIS

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Although there are many references in the literature to conditioned and unconditioned food-induced leukocytosis in man a number of gaps still remain.

One of these is the insufficient investigation of the effect of various sorts of food on these types of leukocytosis. There are only isolated observations in the literature which indicate that leukocytosis is more marked after ingestion of protein than after ingestion of carbohydrates [1, 2 and others]. However, these observations refer only to the so-called digestive leukocytosis which occurs after varying periods of time following intake of food (most often after 2-3 hours).

The references cited say nothing about the relation of conditioned and complex reflex (i.e., occurring within the first 10-15 minutes after the beginning of food ingestion) food-induced leukocytosis to the nature of the food. The present communication attempts to fill this gap at least partially.

EXPERIMENTAL METHOD

The studies of conditioned and complex reflex food leukocytosis were carried out on 46 healthy subjects. A total of 198 investigations of both types of leukocytosis was performed.

The first blood sample for determination of the initial level of leukocytosis was taken 10 minutes after insertion of a Franke needle. White blood cells were then estimated 1-3 minutes after showing food to the subject and the conditioned food leukocytosis was determined from a comparison of the results obtained from the first and second blood specimens.

A number of authors [4, 5] point out that there are two rises of leukocyte count after ingestion of food. The first occurs as early as the 10th-15th minute following food intake, the second 1-3 hours later. Our own observations also confirmed the existence of these peaks. The second rise in most cases was very variable, whereas the first was relatively more marked and constant. This was the reason for taking a specimen of blood for investigation of unconditioned food leukocytosis within the first 15 minutes after the beginning of food ingestion. Taking into account that this leukocytosis was determined not only by unconditioned but also by conditioned factors, it was termed "complex reflex."

Differential leukocyte count as well as total leukocyte count was determined in the majority of subjects, both when food was only shown and when it was ingested.

In order to discover the distinctive feature of conditioned and complex reflex food leukocytosis when various sorts of food were given, the following foodstuffs were used, always in definite amounts (these were assigned definite numbers for convenience): No. 1) 50 g sausage (49 experiments), No. 2) 50 g herring (37 experiments), No. 3)

300 g vegetable soup with meat or fish (30 experiments), No. 4) 100 g white bread (48 experiments) and No. 5) 50 g cake (34 experiments).

EXPERIMENTAL RESULTS

Data indicating the frequency with which the conditioned and complex reflex food leukocytosis occurs in response to the above-mentioned foods are presented first.

Fig. 1 shows, in percentages, the number of examinations in which both types of leukocytosis occurred in response to food stimuli Nos. 1, 2, 3, 4 and 5. The left half of Fig. 1 shows those investigations in which the minimum increase of these types of leukocytosis exceeded 1% with respect to the initial white blood cell level, the right half shows those in which this minimum was greater than 15%.

The curves presented in Fig. 1 indicate that there is certain parallellism between the conditioned and complex reflex food leukocytosis with respect to the extent to which they are marked in response to the various foods (the former type of leukocytosis is in all cases less marked than the latter).

As can be seen from Fig. 1, both forms of leukocytosis are encountered more frequently and are more pronounced in response to sausage, herring and vegetable soup (with meat or fish) than in response to white bread and cake.

Results obtained in some subjects investigated for conditioned and complex-reflex food leukocytosis in response to food stimuli Nos. 1, 2, 4 and 5 are shown by way of illustration in Tables 1 and 2.

TABLE 1

Conditioned Food Leukocytosis

Subjects	Food stimuli											
	№ 1			№ 2			№ 4			№ 5		
	No. of examinations	Present	Absent	No. of examinations	Present	Absent	No. of examinations	Present	Absent	No. of examinations	Present	Absent
I	6	4	2	5	3	2	6	1	5	5	2	3
N	6	5	1	4	3	1	6	4	2	4	2	2
F	6	4	2	4	3	1	6	4	2	4	1	3
B	2	2	—	3	1	2	2	—	2	2	—	2
K	2	2	—	2	1	1	2	—	2	2	—	2
G	2	—	2	2	—	2	2	2	—	2	—	2
K	2	1	1	2	1	1	2	—	2	2	1	1
M	2	1	1	2	1	1	2	—	2	2	—	2

It has been noted above that the first rise in leukocyte count after ingestion of food could be detected in the majority of subjects. This was particularly well shown on comparison of results of white blood counts during the first 15 minutes after the beginning of food intake and 30 minutes after the end of the meal. While in the former case the rise in white blood cell count was very marked, in the latter there was not infrequently even leukopenia. However, in this case too, a definite relation to the nature of ingested food could be observed. Thus, these differences in leukocytosis were present with respect to all the food stimuli except cake, which led to a less pronounced general increase in the number of white blood cells during the first 15 minutes following its ingestion and to relatively more frequent occurrence of increased leukocytosis 30 minutes after its ingestion.

The corresponding experimental results are given in Fig. 2.

Analysis of the data draws attention to the fact that the rise in white blood cells during the first 15 minutes following the ingestion of cake (Fig. 2, 1) is very insignificant compared with the subsequent drop 30 minutes after food intake (Fig. 2, 2). In some subjects the slight complex reflex food leukocytosis in response to cake did not occur until 30 minutes after the end of the meal.

To illustrate these points data (Table 3) obtained from eight subjects given sausage (No. 1) and cake (No. 5) can be used.

TABLE 2

Complex Reflex Food Leukocytosis

Subjects	Food stimuli											
	№ 1			№ 2			№ 4			№ 5		
	No. of examinations	Present	Absent	No. of examinations	Present	Absent	No. of examinations	Present	Absent	No. of examinations	Present	Absent
I	6	5	1	5	4	1	6	4	2	5	3	2
N	6	4	2	4	3	1	6	4	2	4	3	1
F	6	4	2	4	3	1	6	6	—	4	2	2
B	2	2	—	3	3	—	2	1	1	2	1	1
K	2	2	—	2	1	1	2	2	—	2	1	1
G	2	1	1	2	2	—	2	2	—	2	2	—
K	2	1	1	2	1	1	2	—	2	2	1	1
M	2	1	1	Not examined			2	1	1	2	—	2

The question arises whether there are any special features in the differential wbc related to one or other type of food. There are scattered references which state that ingestion of protein food is associated with a predominance of neutrophils while lymphocytes tend to predominate when carbohydrates are ingested [1, 2 etc.].

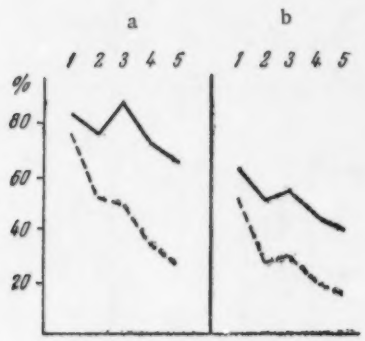

Fig. 1. Relation of leukocytosis to composition of the food——conditioned leukocytosis, - - - - - complex reflex leukocytosis.
1, 2, 3, 4, 5) Food stimuli, sausage, herring, soup, white bread, cake respectively.

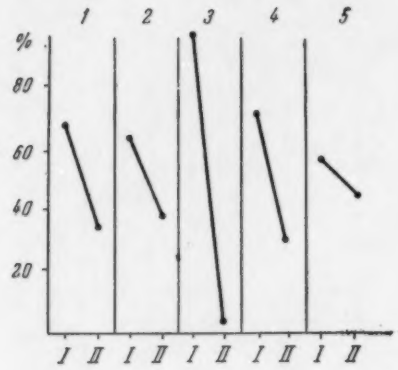

Fig. 2. Dynamics of leukocytosis depending on composition of the food.
1, 2, 3, 4, 5) Food stimuli; I) during ingestion of food; II) 30 minutes after ingestion of food.

In our experiments repeated examinations of the differential wbc in the same subjects (in the absence of food stimuli) showed that the average level of neutrophil and leukocyte fluctuations was most frequently equal to ±5%, the figures for bands being ±2% and for monocytes and eosinophils ±3%.

Table 4 summarizes the experimental results for the different types of white blood cells in subjects given different kinds of food (data on both types of leukocytosis are given for comparison).

When the deviation in the number of individual forms of white cells went beyond the upper and lower limits

of the fluctuations cited above occurring in the absence of food stimuli the observed shifts were correspondingly designated as "increase" or "decrease."

TABLE 3

Number of White Blood Cells During the First 15 Minutes After Beginning of Food Ingestion and 30 Minutes After End of Meal

Subjects	Food stimulus No. 1				Food stimulus No. 2			
	No. of examinations	Presence of complex reflex food leukocytosis	During the first 15 min after ingestion of food	30 minutes after end of meal	No. of examinations	Presence of complex reflex food leukocytosis	During the first 15 min after ingestion of food	30 minutes after end of meal
I	7	6	4	2	5	4	2	2
N	6	5	4	1	4	3	1	2
F	5	4	1	3	4	3	1	2
B	2	2	2	—	2	2	1	1
K	2	2	2	—	2	1	1	—
G	2	1	1	—	2	2	1	1
K	2	1	1	—	2	1	—	1
M	2	1	1	—	2		Absent	

TABLE 4

Increase and Decrease of Individual Types of Leukocytes Depending on Composition of the Food

Food stimulus	Type of leukocytosis	No. of examinations	Segmento-nucleated leukocytes		Band-nucleated leukocytes		Lympho-cytes		Mono-cytes		Eosino-phils	
			increase	Decrease	increase	decrease	increase	decrease	increase	decr.	incr.	decr.
№ 1	Conditioned Complex reflex	45	15	8	—	—	10	14	6	5	3	3
	Conditioned	122	30	29	1	2	34	39	17	14	5	4
№ 2	Complex reflex	38	10	10	—	2	10	9	4	4	4	5
	Conditioned	116	29	31	2	4	34	30	15	10	10	15
№ 3	Complex reflex	23	6	3	—	1	3	8	1	1	3	3
	Conditioned	75	20	15	1	2	21	24	5	3	6	8
№ 4	Complex reflex	50	8	14	1	4	11	8	4	4	4	5
	Conditioned	142	27	47	6	6	44	28	17	18	13	15
№ 5	Complex reflex	32	8	6	—	1	8	10	3	2	2	3
		91	30	14	1	—	14	29	9	5	5	7

It can be seen from Table 4 that ingestion of various kinds of food is accompanied in many cases by slight differences in the number of examinations in which there is increase and decrease of individual types of leukocytes (especially bands, monocytes and eosinophils). Consequently the data obtained agree in some measure with the observations of other authors concerning the predominantly redistributional mechanism of food-induced leukocytosis. At the same time, it is impossible to ignore certain distinctions in the differential wbc when the food ingested is herring and especially white bread on the one hand, and sausage, vegetable soup, and cake on the other. The first two food stimuli elicit an increase of segmented neutrophils and decrease of lymphocytes much less frequently (both on seeing the food and ingesting it). These facts agree with the literature data cited above concerning the nature of differential wbc changes associated with intake of protein and carbohydrate food. There are

indications that lymphocytes contain lipolytic enzymes [3]. This would appear to explain some predominance of lymphocytes observed by us on ingestion of fatty herring.

SUMMARY

The effect of the type of food on leukocytosis was studied in human beings. It was demonstrated that certain foods, such as sausage, herring, vegetable soup cause higher conditioned and complex reflex food leukocytosis than other food stimuli, such as white bread and cakes.

Both types of leukocytosis are redistributional in character. Consequently, it is necessary to take into consideration the character of meals for correct evaluation of results in investigating the conditioned and complex reflex food leukocytosis.

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REFLEX REACTIONS FROM THE STOMACH ON THE BLOOD SYSTEM IN EXPERIMENTAL GASTRITIS

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The primarily redistributive leukocytic reactions which occur during the conditioned reflex phase of digestion are related to the reflex changes in the blood system which occur with the mechanical stimulation of the gastric mucosa. Changes in the number of leukocytes in the peripheral blood have often been observed experimentally in dogs in which the stomach was distended by means of a rubber balloon introduced through a fistula. [1, 5, 6, 8]. Such adequate stimulation of the gastric receptors has usually been observed to cause a rapid, but temporary increase in the number of leukocytes in the peripheral blood, the maximum increase occurring 1-2 hours after the inflation of the balloon; a phase in which the number of leukocytes decreases has sometimes been observed to precede this increase.* The fact that these fluctuations diminish after splenectomy [1] argues their redistributive nature. The reflex character of these changes in the peripheral blood is shown by the fact that they do not occur upon stimulation of gastric mucosa which has been smeared with cocaine. The reflex influx of "white blood" formed elements in large numbers into the organs of the abdominal cavity, which occurs the moment digestion starts, is evidently of definite biological importance, since many enzymes are carried by the leukocytes, especially by the neutrophils and lymphocytes.

Under pathological conditions, however, it is a priori supposed that the normal reflex reactions disappear or become distorted, and that new "pathological" reflexes appear.

Clinical studies have recently been done on the problem of "pathological" reflexes from the stomach on the blood system [2, 3]. However, judging from the literature available to us, there has been no experimental research on this problem. Nor has there been research on how the type and degree of expression of the gastric contractions affect these reflexes.

Our purpose in this work was to ascertain the nature of the reflex reactions from the stomach on the blood system under conditions of experimental gastritis, using data showing the changes in the leukocyte ratio and in the number of leukocytes in the peripheral blood.

EXPERIMENTAL METHODS

The experiments were done on 2 dogs with gastric fistulas. We used a rubber balloon filled with 500 ml of air to cause mechanical adequate stimulation of the gastric mucosa. The inflated balloon was left in the stomach for 3 hours. Blood was collected in a mélangeur from an incision made in the inner surface of the cochlea before the inflation of the balloon, and then 5 minutes, 30 minutes, 1 hour, 2 hours and 3 hours after the inflation. Two blood smears were taken – one before the inflation of the balloon and one 2 hours after the inflation.

* The author considers the use of the terms "digestive leukocytosis" and "digestive leukopenia" incorrect in this case, as it is a question of the relative fluctuations in the number of leukocytes as compared with a given original level.

The motor activity of the stomach was recorded with a manometrograph during the first 1½ hours of the experiment.

We used the method used in the Pavlov Laboratories to obtain experimental gastritis [4]: brief irrigation of the gastric mucosa with 1000 ml of a 10% solution of silver nitrate. One irrigation was done on the dog Reks and two, one month apart, on the dog Dichka. The mild inflammation thus induced can be examined as a model of the exogeneous form of acute gastritis [7]. The general condition of the dogs was not much changed after the irrigation of the gastric mucosa. Some vomiting was observed on the day of the irrigation, and subsequently a large amount of mucus was discharged from the fistula for a period of 7-10 days. The alimentary excitability of the animals did not suffer.

The experiments were conducted 14 and 15 hours after feeding, in an isolated chamber under uniform conditions.

Studying the leukocytic reflex reactions in dogs is complicated by the fact that there are considerable fluctuations in the leukocyte content of the peripheral blood of the animal under normal conditions [8, 9]. Because of this fact, 86 experiments were done on the two dogs over a period of 4 months to study the normal fluctuation range. Blood was taken at the same time intervals as in the main experiments with stimulation of the gastric receptors; the dogs were on the bench for a period of 2-3 hours.

EXPERIMENTAL RESULTS

It was established that the leukocyte content in 1 mm^3 of peripheral blood was subject in the control dogs to considerable fluctuations, both in the course of a single experiment and on different days. The extent of these fluctuations was as follows for the whole experimental period: from 6300 to 17,000 cells per 1 mm^3 in Reks, and from 6300 to 18,000 in Dichka. The difference between the largest and smallest number of leukocytes per 1 mm^3 during a single experiment was 400-8200 cells in Reks and 500-8300 cells in Dichka. The fluctuations of the leukocyte level during one experiment lessened considerably towards the end of the 4-month period, which fact seems to have been due to the development of extinctive inhibition to external stimuli. In comparison with the original level (at the start of the experiment), the number of leukocytes in the samples of blood taken subsequently, during the experiment, both decreased and increased; an increased number was more frequently observed (in Reks, 32 times out of 44 experiments, and 37 times out of 42 experiments in Dichka). The degree of increase was not great. Comparing the highest leukocyte content in a given experiment with the original level, we found the average increase to be 26.2% for Reks and 26.3% for Dichka. The leukocyte content could be highest during the beginning, middle or end of the experiment.

Our 53 experiments with the stimulation of normal gastric mucosa followed the basic pattern observed by most researchers – the leukocyte content increased after the balloon was inflated in the stomach. The average increase in the number of leukocytes was 55.4% in Reks and 57.9% in Dichka. In Reks the leukocyte level increased in all 25 experiments, in Dichka, in 25 out of 28 experiments. In experiments with a high initial leukocytosis, a pronounced decrease in the number of leukocytes was observed in all the blood specimens taken after the gastric stimulation.

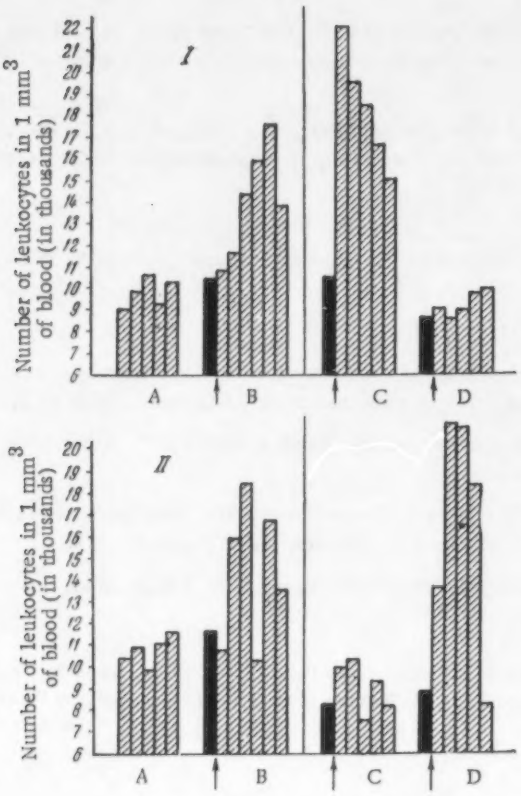
The highest leukocyte level was observed 30 minutes, 1 hour, 1 hour and 30 minutes and 2 hours after the inflation of the balloon.

We did not observe the initial "leukopenic" phase which has been described by several authors. The degree of decrease in the number of leukocytes after inflation of the balloon was not usually high in comparison with the original level (except for cases with a high original leukocytosis), and the decrease occurred at different times, as in the control experiments.

High leukocytosis, which persisted after inflation of the balloon, was observed on the 2nd day after the introduction of the silver nitrate solution.

With the further development of the experimental gastritis, two alternating forms of reflex leukocytic reactions were observed in the dogs in response to the gastric stimulation: 1) reaction weakly expressed or absent – the fluctuations in the number of leukocytes after inflation of the balloon, as compared with the original level, did not exceed the fluctuations in the majority of the first series of control experiments; this type of reaction was usually observed during the first 3-4 days; 2) reaction sharply expressed – high leukocytosis (20,000 - 23,000 cells

per 1 mm^3), which did not occur in the experiments with the stimulation of normal mucosa, was observed after the inflation of the balloon.



Changes in the leukocyte count in the peripheral blood of the dogs Dichka (I) and Reks (II). A) Without the use of stimuli; B) with stimulation of the normal gastric mucosa; C, D) stomach stimulated during the first 2 weeks in the development of gastritis. Black columns – original level of leukocyte count before inflation; arrow – moment of inflation.

These two forms of the reaction, the disappearance and the intensification of the reflex, occurred alternately for a period of one month after the burn in 8 out of 12 experiments on Reks and in 10 out of 12 on Dichka. As the pathological process abated, the normal type of reflex was reestablished, but again became distorted in Dichka after the second use of the silver nitrate.

Examples of normal reflex leukocytic reactions and their changes in experimental gastritis are shown in the graph.

Therefore, our experimental data agrees with the clinical observations which reported the intensification of reflex leukocytosis attending gastric stimulation in patients with hyperacid gastritis and the lack of a reaction with the hypacid form of gastritis [2]. In ulceration patients the reflex was even observed to be distorted [3].

The initial differential wbc had not changed much 2 hours after the start of the balloon inflation in the two dogs. Usually, a slight redistribution of the percentage content of segmented neutrophils and lymphocytes had occurred in the direction of a predominance of these or other cells. Less frequently (in 11 out of 27 experiments), a noticeable increase (7-11%) in the number of neutrophils was observed; in 6 of these 11 experiments, there was a shift to the left in the leukocyte ratio, i.e, the number of stab cells increased. High leukocytosis was not always attended by changes such as these, but could occur without any noticeable changes in the leukocyte ratio. This indicates that this leukocytosis is primarily redistributive in nature. Accelerated leukopoiesis does not seem to be the main factor behind the leukocytosis which occurs during the conditioned reflex phase of digestion.

After the introduction of the silver nitrate solution, the changes observed in the differential wbc of both dogs were extremely slight, both before and after stimulation. Neutrophilia and shift to the left were not observed even in cases with very high leukocytosis. It is difficult to explain these facts without morphological examination of the bone marrow.

After comparing the degree to which the motor activity of the stomach was expressed with the nature of the leukocyte reflex both before and after the stimulation, we were unable to establish any correlation between the two indices; a complete lack of any leukocytic reaction could be combined with pronounced gastric motor activity, or, conversely, high leukocytosis could be found after the inflation of the balloon with gastric motor activity completely inhibited. This again indicates the reflex nature of the changes which occur in the peripheral blood with stimulation of the gastric interoceptors. The mechanical factor (pressing blood from the spleen) does not play an important part.

The pathogenesis of acute and chronic gastritis is one of the problems of internal medicine. It is of definite value to the study of this problem to show the type of changes which occur in the reflex reactions of the affected organ. The changes which we have described in the leukocytic reflex during experimental gastritis evidently depend primarily upon the implication of the cardiovascular system in the pathologic reaction, since the leukocytic reactions are primarily redistributive.

SUMMARY

It was established that considerable reflex changes of the content of peripheral blood occur in dogs under conditions of experimentally induced gastritis. The reflexes were observed to disappear or to become intensified depending on conditions of the experiment.

Reflex changes in the content of the peripheral blood were observed in dogs as a result of adequate mechanical stimulation of the gastric receptors: the leukocyte count increases due to changed vascular redistributive reactions.

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REFLEXES FROM THE INTESTINE ON THE LYMPH FLOW IN ONTOGENESIS

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Morphologists and physiologists have obtained valuable data in recent years concerning the innervation and functional regulation of the lymphatic system. According to the literary data [13], the reflex which causes the lymph vessels to contract is a contributory cause of edema in thrombophlebitis, since it hinders the elimination of the transudate through the lymph vessels. There are indications that the lymph circulation is disturbed in cardiac edema, that the lymph circulation plays an important part in the function of different parenchymatous organs [13] and that the lymphatic system participates in hypertonic disease [12].

The data adduced shows that the blood and lymph circulatory systems complement each other, together constituting a whole, and therefore that the physiological study of lymph circulation is of definite clinical importance.

The problem of lymph flow regulation during postnatal ontogenesis has been studied for several years at the Kazakh Medical Institute's Department of Normal Physiology. In the course of this research, we discovered certain age peculiarities in the reflex regulation of the lymph flow [1-4]. We are now experimenting to find the age peculiarities in the interoceptive reflexes on the lymph flow.

The existence of reflexes from the internal organs has been demonstrated in many works by Russian physiologists. Reflexes from the baroreceptors and chemoreceptors of the organs of the abdominal and thoracic cavities on arterial pressure and respiration have been proven to exist [17]. Interoceptive influences on the lymph flow have been established from the intestines, spleen, kidneys and kidney pelvis [8]. Reflexes from the same internal organs on venous pressure have been discovered [14-16].

There have recently been in the literature data concerning the interoceptive influences from the internal organs in ontogenesis. For example, L. E. Pal'gova [9-11] demonstrated in our laboratory the characteristics of the reflexes from the baroreceptors and chemoreceptors of the intestine, spleen and the excretory organs on arterial pressure and respiration in ontogenesis. E. I. Komarov [5-7] has described the reflexes from the mechanoreceptors and chemoreceptors of the bladder and intestine in kittens and puppies. We could find no literary information on the question of interoceptive influences on the lymph flow in ontogenesis.

This article presents the results obtained from some of our experiments with the stimulation of the intestinal mechanoreceptors in puppies of various ages to find the effect of this stimulation on the lymph flow.

EXPERIMENTAL METHODS

In order to stimulate the intestinal mechanoreceptors, we distended the walls of an isolated intestinal loop by inflation. Intestinal inflation is the most adequate stimulation, since it is a frequent symptom of various intestinal diseases.

The experiments were conducted in the following manner. The animals were fixed to the operating table under ether anesthesia, and we operated to produce a fistula of the thoracic duct. Without dwelling on the details

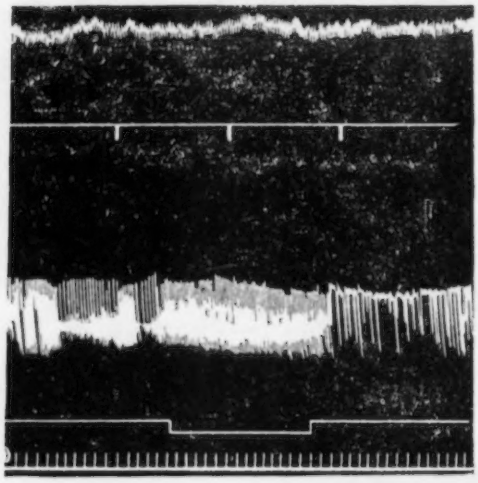

Fig. 1. Change in the lymph flow in an experiment on a puppy 10 days old.
Curves (from the top to bottom) show: arterial pressure, lymph flow, respiration, indication of stimulation, indication of time (in 3 second marks).

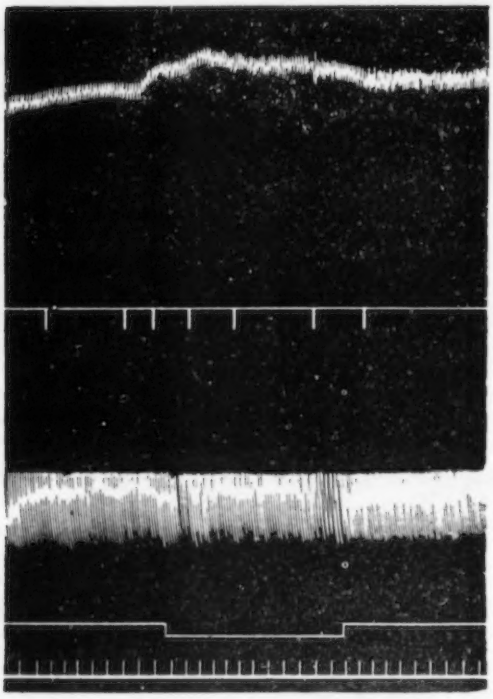

Fig. 2. Change in the lymph flow in an experiment on a puppy 14 days old.
Curves are the same as in Fig. 1.

of the operation, we wish to note that it was not completed, i.e., the drainage cannula was not inserted, so that the lymph was not discharged in vain. After the anesthesia had passed, we ligated the carotid artery and inserted a cannula in the trachea. Then the abdominal cavity was opened by means of a small incision, and a loop of the small intestine withdrawn. A section of the intestine 10-15 cm long was excised between two ligatures, so that its only connection with the body was the neurovascular plexus which passes into the mesentery. Air was fed into the intestinal lumen through a cannula attached at one end of the isolated intestinal loop and connected by a rubber tube to a balloon. The amount of pressure produced was regulated by a mercury manometer. The intestinal loop was wrapped with gauze, which was moistened from time to time with a warm physiological solution. Then the jugular vein was dissected out in the region where the thoracic duct empties into it, and a cannula was inserted into the vein.

We recorded the lymph flow, arterial pressure, respiration, periods of stimulation and the time in 3-second intervals while the intestinal mechanoreceptors were being stimulated in this way by inflation.

EXPERIMENTAL RESULTS

We conducted 112 experiments on 30 puppies of varying ages. Inflation of the isolated intestinal loop with a pressure of 50-80 mm of mercury did not cause any pronounced changes in the lymph flow of puppies aged up to 14-16 days; in some cases, the lymph flow was even observed to decrease. There were various changes in the respiration of these puppies; the respiratory movements became either faster and deeper or slower and deeper. No great changes were observed in the arterial pressure. Sometimes a slight increase was observed (Fig. 1). In puppies aged 14-16 days or more, the same stimulation caused a pronounced increase in the lymph flow on a background of the same respiratory changes. There was a slight, gradual increase in arterial pressure. A pressodepressor reaction was observed in some cases (Fig. 2).

Upon analysis of the data obtained, we discovered that the change in the lymph flow depended on the age of the experimental animals. In puppies up to 2 weeks old, stimulation of the intestinal mechanoreceptors did not cause changes in the lymph flow, although there were changes in the respiration and sometimes in the arterial pressure; if changes in the lymph flow did occur, as we observed

in some experiments, they were characterized by a decrease in the lymph flow, unlike the changes which occurred in the puppies more than 2 weeks old (we did not observe an increase in the lymph flow in any of the experiments on the younger age group). In puppies more than 2 weeks old, however, we observed an increase in the lymph flow in all the experiments, while the changes in arterial pressure and respiration were the same as in the younger age group. Moreover, the increase in the lymph flow occurred whatever the nature of the changes which occurred in the respiration and arterial pressure.

The fact that, in most of our experiments on puppies less than 2 weeks old, no changes occurred in the lymph flow in spite of the existence of functioning mechanoreceptors in the intestinal wall (shown by the blood pressure and respiratory reactions) may possibly be explained on the basis of data which we obtained earlier concerning the development of vagus reflexes on the lymph flow in growing puppies [4]. We showed that in newborn puppies, unlike mature animals, stimulation of the central and peripheral portions of the vagus nerve causes changes in the arterial pressure and respiration, but none in the lymph flow. This could indicate that the innervation of the thoracic duct is not completely developed in newborn animals.

The data presented indicate , first, that the intestinal mechanoreceptors can influence the lymph flow in pappies aged 2 weeks or more, and second, that this is a reflex type of reaction. The latter is shown by the fact that, in our experiments, the lymph flow increased in experimental animals 2 weeks old as soon as the stimulation was applied, as well as by the fact that the lymph flow did not change in animals less than 2 weeks old, although there were changes in the arterial pressure and respiration.

SUMMARY

Experiments were performed in 30 puppies. It was shown that the reflex regulation of lymph circulation depended on the age. Stimulation of gastric mechanoceptors caused no change of the lymph flow in puppies up to 14 days old. However, in puppies older than 14 days increased lymph flow was noted with stimulation of the intestinal mechanoreceptors.

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ON THE RELATIONSHIP BETWEEN THE FUNCTIONAL AND STRUCTURAL DEVELOPMENT OF THE CEREBRAL CORTEX IN THE PROCESS OF ONTOGENESIS

COMMUNICATION I. THE ONTOGENIC DEVELOPMENT OF CONDITIONED REFLEX ACTIVITY IN RABBITS

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We undertook to find the relationship between the functional and structural development of the cerebral cortex in rabbits in the course of ontogenesis. This problem was solved by comparing the data obtained from physiological and histological studies.

In this article, we present the results of experiments made to determine the earliest time during postnatal development that it is possible to produce conditioned reflexes in rabbits.

In order to obtain data as conclusive as possible, we used both the food and defense methods of producing conditioned reflexes; a conditioned reflex was developed to olfactory, sound, tactile, thermal and light stimuli.

EXPERIMENTAL METHODS

The food conditioned reflexes were developed according to V. A. Troshikhin's method [5], so modified that it was possible to develop conditioned reflexes from other analysors as well as the olfactory and that the reflexes could be recorded as objectively as possible.

Several days before parturition, the mother rabbit was put into a large, specially made box, with two compartments – a nursery compartment, where the mother rabbit made a nest and where the baby rabbits were born, and a compartment for the mother rabbit. The first compartment of the box had a movable floor, placed on four rubber balloons which were attached to a Marey's recording cylinder. This arrangement permitted the fluctuations of the floor caused by the movements of the rabbits in the nest to be recorded on a kymograph.

At the same time, the rabbits were watched through a specially made small window, so that their reactions could be described in detail.

The mother rabbit nursed the young rabbits once a day. She was removed from the nest every evening so that the baby rabbits might learn to associate their feeding with the action of the various conditioned stimuli. When the mother was put back into the nursery on the following morning, she began at once to feed the baby rabbits. Experiments with the development of conditioned reflexes were done on 13 litters of rabbits.

In order to develop the conditioned reflexes, the mother's nipples were smeared with some sort of odiferous substance (eau de cologne, camphorated oil, etc.) before nursing.

The experiments showed that this type of conditioned reflex develops very quickly – during the very first feeding. These data agree with the results of similar studies conducted on puppies by V. A. Troshikhin [5].

The conditioned reflex could be determined in two ways. 1) When a conditioned reflex was present and food excitability was sufficiently high, the following typical reaction was caused in the rabbits by the introduction into the nest of a cotton wad dipped in the odiferous substance. The rabbits went into a state of great motor excitement, which was manifested by complex movements of their heads and bodies (resembling those which are performed when the rabbits are searching for their mother's nipples), by their mouths opening and making sucking motions while they lapped and rolled over on their backs, and finally by the characteristic action of "licking their chops", which we believe to be one of the most specific signs of the food reflex. 2) A baby rabbit was taken out of the nest, and a palm smeared with the appropriate odiferous substance was held up to it. When a conditioned reflex was present, this action caused great excitement in the rabbit; he crawled up to the palm, raised his head and began to lick the hand, moving his head as though looking for the nipple. The most typical reaction was the rolling over of the rabbit on its back, as rabbits naturally nurse lying on their backs (Fig. 1).

Fig. 1. Conditioned food reflex to smell. A palm smeared with "Chypre" eau de cologne, the odor of which was a conditioned food stimulus. Rabbits rolling over on their backs in the typical fashion.

Fig. 2. Conditioned food reflex to air jet. Rabbit sucking the tip of a bulb producing the air jet.

In control experiments, it was established that the use of the same stimuli before the development of the conditioned reflexes did not cause the typical food reaction described above and sometimes caused a negative motor reaction.

Experiments with the development of a conditioned reflex from the skin analysor were done on 10 litters of rabbits; 8 of these 10 litters were used to develop a reflex to tactile stimulation, and 2 to develop a reflex to thermal stimulation.

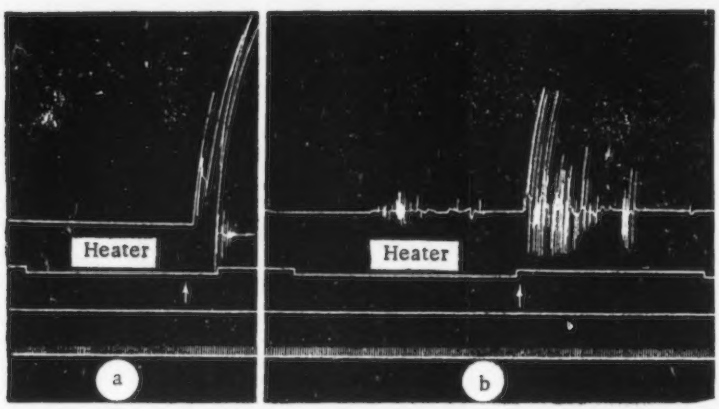

Fig. 3. Kymograms of experiments with the development of a conditioned food reflex to heat.
a) 10th experiment, no reflex; b) 11th experiment, expressed food reflex. Curves (from top to bottom) show: recording of rabbits' movements, indication of conditioned stimulus (heater); ↑ – unconditioned stimulus (mother's arrival and feeding), indication of time in 1-second marks. Sharp rise in the motion curve – arrival of mother in nest and beginning of nursing.

To develop a conditioned reflex from the skin analysor, an air jet (tactile stimulus) was blown into the nest before feeding, by means of a rubber bulb or electroventilator, or a special heater was put into the nest (thermal stimulus). In the first experiments, the conditioned stimulus was used first for 10-15 seconds, then the time was increased to 30 seconds and in some experiments to 1 minute.

In the various series of experiments, the conditioned stimulus was either used for 10-15 seconds during feeding, as in the majority of cases, or for the entire feeding period (3-5 minutes).

After the conditioned reflex had developed, the use of these stimuli alone caused the typical motor reaction – great general excitement in the rabbits. The rabbits ran about the nest as though looking for their mother, made sucking motions, "licked their chops" and crowded up to the place where the conditioned stimulus was being produced. When the air jet was being used, for example, the rabbits gathered at its source and sometimes even sucked at the tube from which the air issued (Fig. 2). When the heater was being used, the rabbits licked the screen partitioning the heater from the nest. These observations can be compared with the known fact that a dog will lick a lamp, the light of which is a conditioned food stimulus for it.

The food reaction described could also be reproduced outside the nest; in some of the experiments, we used a motion picture camera to record the reaction.

The presence of feeding motions (sucking, lapping and chop licking), as well as the stability and force of the food reaction clearly distinguished this reflex from other types of reactions, the orientation reflex, for example, which was observed with the first uses of the conditioned stimulus or with the use of other stimuli.

Experiments developing a conditioned reflex to sound were done on 11 litters of rabbits. To develop this conditioned reflex, a sound signal was turned on before feeding (electric oscillator, 500 oscillations per second). The experimental conditions and the nature of the conditioned-reflex food reaction were the same as those described with the development of a conditioned reflex from the skin analysor.

We did not conduct specific experiments with the development of conditioned food reflexes to light, but a conditioned food reflex of this type did develop in the rabbits at a definite age, since their nest was lighted before every experiment.

To ascertain the stage in postnatal development when conditioned reflexes begin to develop, we conducted experiments from the day the rabbits were born. One experiment was done a day on each litter (the mother rabbit suckled the litter only once a day). A reflex was developed to one stimulus at each experimental stage.

The method we selected allowed the conditioned reflexes to develop under conditions as physiological as possible. The possibility of external inhibition was almost completely excluded, since the rabbits were not taken out of the nest. We used a reaction natural to the rabbits (looking for the mother's nipple) as the conditioned reflex reaction. All these factors favored the rapid development of stable conditioned reflexes, which made it possible to determine fairly exactly the time at which the corresponding analysor became functionally mature. The disadvantage of the method we used was the impossibility of making an exact quantitative evaluation of the conditioned reflex. Moreover, the kymographic recording only showed the reaction of the litter as a whole.

Besides the food conditioned reflexes, conditioned defense reflexes to sound and light stimuli were also developed in the rabbits. The method used for this purpose was analogous to that described by A. B. Kogan and S. N. Shchitov [2].

EXPERIMENTAL RESULTS

The experiments conducted showed that there were differences of 1-2 days in the times at which the conditioned reflexes developed in both the different litters of rabbits and the different rabbits of one litter. The data presented for each experimental series are the average for all the experiments.

Conditioned food reflexes to olfactory stimuli could be developed from the first day after birth.

The conditioned food reflexes from the skin analysor to both the tactile and thermal stimuli began to appear the 10th day after birth (Fig. 3). The conditioned reflexes were still unstable and did not always appear at this stage; the most pronounced aspect of the reaction was that pertaining strictly to food (sucking motions, et al.), while the general motor reaction was more weakly expressed. The reaction only began to acquire greater stability on the 13th day after the rabbit's birth, when all the aspects of the reaction became clearly expressed. Condi-

tioned food reflexes to sound stimuli began to develop 10-11 days after birth, becoming more stable the 13th day.

The defense conditioned reflexes to sound began to develop at about the same time as the food conditioned reflexes, i.e., 10 days after birth.

These data agree with the results obtained by A. A. Volokhov [1] and G. A. Obraztsova [4], who studied the development of a conditioned rejection (shaking off) reflex to sound in rabbits.

Conditioned food reflexes to light stimuli began to develop on the 13th day after birth (reflexes unstable). More stable reflexes began to develop the 15th day. These data agree with the results of the experiments conducted with the defense method.

Unconditioned food and defense reflexes appeared in the rabbits from the moment of birth. Orientation reactions to the tactile, sound and light stimuli appeared before conditioned reflexes could be developed to these stimuli (on the 1st, 7th and 10th days after birth, respectively). The experiments show that the times at which conditioned reflexes develop depend on the times at which the cortical centers of the appropriate analysors, which mature later than the subcortical formations, achieve functional maturity; this fact agrees with data obtained from research on puppies [3, 6].

SUMMARY

It was established in experiments on rabbits that conditioned food reflexes to olfactory stimulation commence to develop from the very first day after birth. Conditioned food reflexes to tactile, thermal and sound stimulations develop from the 10th to 13th day. Reflexes to light stimulations begin to develop from the 13th to 15th day after birth. Conditioned defense reflexes to sound and light develop at the same periods as the conditioned food reflexes to these stimulants.

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CHARACTERISTICS OF SLEEP AND WAKING ELECTROENCEPHALOGRAMS IN DOGS OF DIFFERENT AGES

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The question of the special features of the electroencephalogram (EEG) at early ages has not yet been solved exhaustively, despite the fact that there is a number of investigations dealing with the characteristics of cerebral cortical activity both in animals of different ages [3, 5, 6, 10] and in children of different ages.

Two points of view have emerged in connection with the special EEG features in children.

One view, postulated by D. Lindsley [16] regards the whole frequency spectrum from 3 to 13 cps, as recorded from children of different ages, as an expression of gradual establishment and reorganization of the same electrical activity, viz., the alpha-rhythm. The latter appears in children at the age of about 3 months at a frequency of 3-4 cps and only attains the frequency of 10-12 cps by the age of 13-16 years. This viewpoint is shared by a number of authors [15, 18].

The other view is represented by L. Cornil [14] and his collaborators. These authors consider that the transition of electrical activity from 3-4 cps in very young children to the adult alpha-rhythm as noted by Lindsley is spurious. Cornil et al. suggest that the various rhythms (subdelta, delta, theta and alpha) in children's EEG exist simultaneously but their preponderance varies depending on the age.

In the USSR this point of view appears to be subscribed to by A. B. Kogan and N. V. Shteinbukh [7].

The present work is concerned with obtaining the chracteristics of natural sleep and waking electroencephalograms recorded from dogs from the first days of life to adult age.

EXPERIMENTAL METHOD

Experiments were performed on dogs of various ages. The animals were not fixed in the stand and were free to assume natural postures. Newborn pups were subjected to experiments immediately after breast-feeding, older pups were given additional food just before the experiment.

Electroencephalograms were recorded by the bipolar method, using needle electrodes inserted into the cranial bone in the frontal or parietal regions. The interelectrode distances was 1-1.5 cm. Simultaneous EEG and electrocardiogram (ECG) records were made on a two-channel ink-writing electroencephalograph.

The amplifier range band was 1 to 100 cps. Extension of this to include lower frequencies (0.5-0.2 cps) complicates EEG recording from a nonfixed animal. Amplification 1 cm = 50 μv.

Repeat EEG recording from the same pup at a very early age presented the difficulty that insertion of needle electrodes into the cranial bone could only be done episodically. Special observations showed that the use of such electrodes daily or even every other day caused the appearance of chronic inflammation in the cranial bones. On the other hand, it is impossible to apply the in-dwelling electrode technique to pups only a few hours or a few days

old. Therefore we made use of single or episodic insertion of needle electrodes in most of our experiments.

A total of 160 pups of various ages was subjected to investigation under different experimental conditions.

EXPERIMENTAL RESULTS

The investigations permit the conclusion that the waking state and natural sleep have their chracteristic EEG features at various ages.

In adult dogs and pups aged over 3 months the dominant activity in the waking state is of low voltage (20--25 μv) with a frequency of 45 cps. The lower limit of frequency is 35-40 cps, the upper 50-60 cps. This rhythm can naturally be described as corresponding to β-rhythm. On transition to sleep the background activity disappears, "spindle" activity (10-12 cps) appears during drowsiness and so-called "sleep" potentials (slow waves at 3-4 cps and up to 100 μv in amplitude) appear in deep sleep. Similar electroencephalographic events have been described during sleep and waking by many authors, both Soviet and foreign [4, 9, 11, 12, 17].

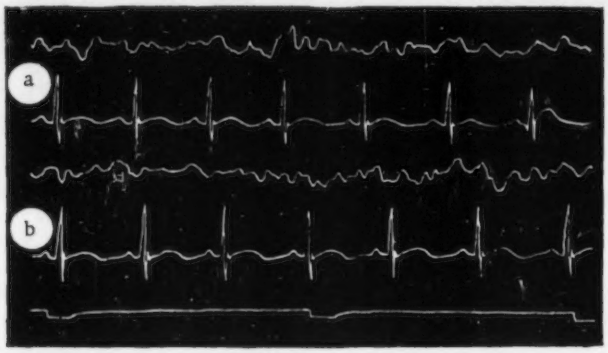

Fig. 1. Electroencephalogram and electrocardiogram of a 7-day-old pup.
a) Waking state; b) during natural sleep.

Investigations carried out on very young pups indicate that the frequency and amplitude characteristics found in the EEG of adult animals during waking and natural sleep, as well as those changes observed during transition from one to the other, are absent during the early stages of postnatal ontogenesis. Pups aged up to 16-18 days show bioelectric cortical activity in the waking state which consists of more or less regular, and in a number of cases dominant, activity at 10-14 cps and amplitude which rarely exceeds 15μv. The lower limit of this activity may be 6-8 cps. Unlike this, slow activity at 3-4 cps and of unstable amplitude (10-50 μv) is less constant and may even be episodic. At the same time this slow rhythm is characteristic for EEG of young animals in the waking state (Fig. 1, a).

It is known that in the newborn the periods of sleep greatly exceed those of waking, which only occur during feeding. Observations made in our laboratory have shown that each succeeding waking results from excitation of the feeding center by the "fasting" composition of the blood.

Allowing the newborn to take milk in the amount equal to the capacity of his stomach elicits reflex inhibition of the feeding center with transition to general sleep [1, 8].

EEG recorded from very young pups during deep natural sleep revealed no appreciable change in the frequency of the bioelectric activity (Fig. 1, b) . In some cases a drop in frequency of 1-2 cps can be seen and decrease in amplitude is noted somewhat more commonly, but these changes are not regular and consistent and may occur both on transition from sleep to wakefulness and vice versa. Both in the very young pups and at later stages the transition to a stage of sleep is accompanied by some decrease in the rate of cardiac contractions (Fig. 1, a and b).

The significant finding is the absence in the very young age group of a difference between the electrical activity of the cerebral cortex during wakefulness and in sleep.

The more or less regular and rhythmic activity at 10-14 cps described above must be formally designated

as activity of alpha-rhythm type. But, unlike the alpha-rhythm of adult animals, this activity is not desynchronized on transition to the waking state. Further analysis of the evolution of this activity suggests rather that it corresponds to the future beta-rhythm of adult animals. Thus, beginning from the 18th-20th day of life, the main and predominant waking activity is a rhythm at 16-18 cps; by the age of one month such activity is at 20-22 cps. Slow activity at this age is exceptional during wakefulness (Fig. 2, a).

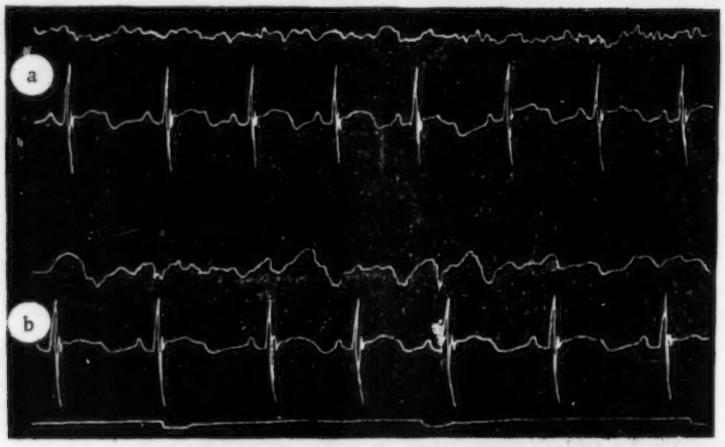

Fig. 2. Electroencephalogram and electrocardiogram of 18-day-old pup.
a) In the waking state; b) during natural sleep.

The dominant activity mentioned above increases in frequency with age and by the age of 3 months reaches frequencies of the β range as observed in adult animals in the waking state (Fig. 3, a). Concurrently, beginning with the age of 18-20 days, the transition to sleep is accompanied by the appearance of slow "sleep" potentials with frequencies of 4-5 cps and of considerable amplitude (Fig. 2, b). Slow "sleep" potentials become more prominent with age and by the age of 3 months approach those of adult animals in amplitude and frequency (Fig. 3, b and c).

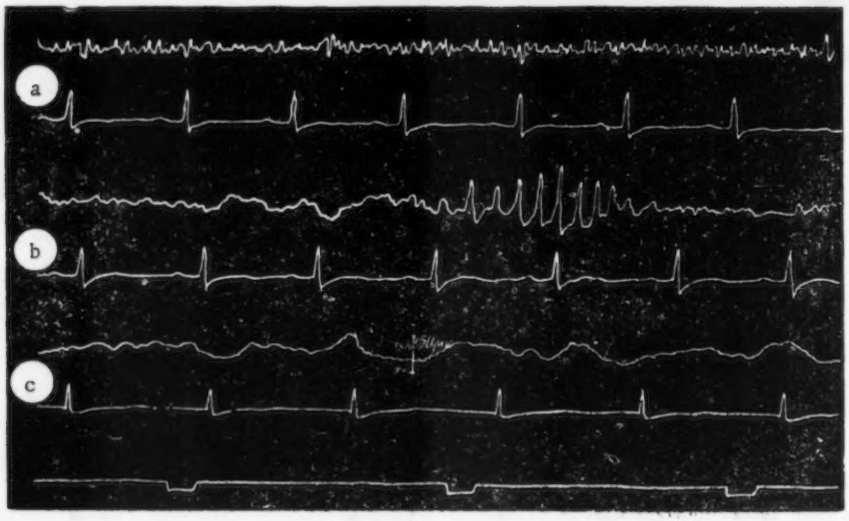

Fig. 3. Electroencephalogram and electrocardiogram of 3-month -old pup.
a) In the waking state; b) during drowsiness; c) during natural sleep.

According to the data obtained in our laboratory the onset of sleep is associated with the development of the second catelectrotonic parabiotic process in the nuclei of the thalamic region which play the part of an intermediate link with respect to afferent impulses passing to the cerebral cortex [2].

In modern interpretation such an intermediate link is constituted by the nonspecific nuclei of the thalamic reticular system. The pessimal state with signs of negativity and depolarization developing in these nuclei on the one hand blocks those constant influences which pass, during wakefulness, from these nuclei to the cortex, and on the other hand elicits a state of heightened polarization, i.e., electropositivity, in the cortical cells; this occurs through perielectrotonic contrast. Such a concept of the mechanism of the onset of sleep approaches in some aspects only to the corresponding views of F. Bremer [13].

I. A. Arshavskii considers that the nonspecific nuclei of the thalamic reticular system play the same role of intermediate link with respect to the cerebral cortical cells as the sinoauricular node with respect to myocardial elements or the neuromuscular junction with respect to the skeletal muscle elements.

The data presented suggest that the absence of difference in the special features of the cerebral cortical electric activity during wakefulness and sleep in very young pups is naturally explicable by the absence of functional, and evidently structural, maturation of the appropriate nuclei of the thalamic reticular system, viz., its ascending part. The first signs of such maturation are detected in pups beginning with the 18th-20th day of life. This serves to explain why it is at this very age that the electrical activity shows an appreciable drop in frequency during sleep. This is also associated with a rise in the upper limit of frequency of the cerebral cortical electrical activity during wakefulness.

SUMMARY

Every age-period is characterized by definite frequency and amplitude indices of EEG, depending on the state of the animal.

In young animals the basic electrical activity, when the animal is awake, is characterized by a rhythm of 10-14 cycles per second. It corresponds to the future rhythm of β-type. Commencing from the age of 18 days the rhythm increase to 16-18 per second while at 3 months it equals 35-45 per second.

Sleep does not cause any significant changes in the bioelectric activity of young puppies. The first signs of change of EEG in sleep (as compared to that while the dog is awake) commence from the 18-20th day of the dog's life and are manifested by the appearance of slow oscillations of large amplitude. When the puppies are 3 months old the typical changes in the EEG, characteristic of adult animals, appear in sleep.

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PARTICIPATION OF AN INTEROCEPTIVE COMPONENT IN THE MECHANISM OF ALTERED KNEE JERK UPON INTRAVASCULAR ADMINISTRATION OF HYPERTONIC SOLUTION

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Substances administered intravenously inevitably come in contact with the tissues of the heart and lungs.

Numerous physiological [7, 8, 9] and morphological [1, 2, 3, 7] investigations show that the heart and lungs are a vast receptor field, extremely sensitive to chemical and mechanical stimuli.

We succeeded in showing earlier [5] that changes in the cortical motor response and the knee jerk reflex were much more pronounced on intravenous administration of hypertonic sodium chloride solution than when the latter was supplied directly to the brain by way of the common carotid artery. The same had been observed in other investigations [4].

The present work attempts to compare the changes in the knee jerk which arise upon administration of hypertonic NaCl solution into the heart with changes occurring when the solution is supplied to the spinal cord by way of the aorta in order to evaluate the role of the cardiac-pulmonary receptor field in the mechanism of action of the intravenously injected substance on the spinal reflex.

EXPERIMENTAL METHOD

The work was carried out in short-term experiments on cats under intravenous urethane anesthesia (1 g/kg). A fine, elastic chlorovinyl catheter was passed through the femoral or the external jugular vein into the right auricle and through the femoral or left carotid artery into the various parts of the aorta (arch, thoracic and abdominal). The knee jerk was elicited by automatic rhythmic strokes of a hammer of standard strength and frequency of once per 2 seconds, applied to the tendon of the quadriceps femoris muscle. The limb was fixed in a rack by means of a pin through the lower third of the femur.

During the experiments the following were recorded: blood pressure in the common carotid artery, respiration in the trachea, knee reflex (extension of the shin) with the help of two Marey's tambours.

Hypertonic (20%) NaCl solution was introduced through the catheter in amounts of 1-4 ml at a rate of 1 ml in 2-3 seconds. The catheters were first completely filled with hypertonic solution.

EXPERIMENTAL RESULTS

Preliminary experiments established that similar inhibition of the knee jerk reflex occurred in response to intravenous administration of hypertonic NaCl solution through a cannula in the femoral vein and through a catheter passed through the contralateral femoral or external jugular vein into the right auricle. Of 60 introductions of hypertonic saline solution into the right auricle through the catheter, in 51 cases inhibition of the knee jerk was elicited which was similar in intensity and duration to that elicited by injection into the femoral vein.

* Deceased.

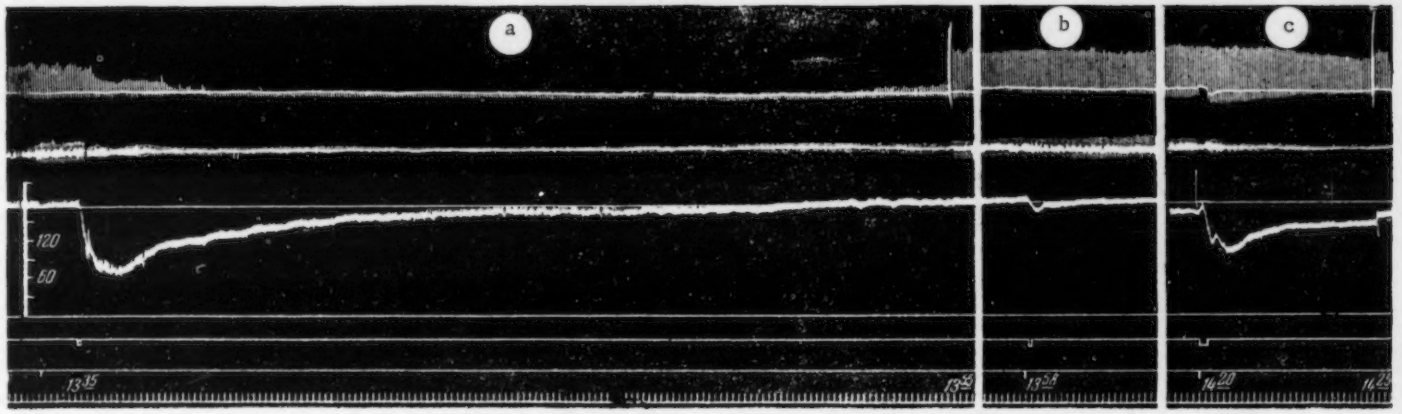

Fig. 1. Changes in knee jerk reflex in response to administration of 1 ml 20% NaCl solution into the right auricle (a) and arch of the aorta (b) and 4 ml 20% NaCl solution into the arch of the aorta (c) (cat under urethane anesthesia).

Records from above down: knee jerk, respiration in trachea, arterial blood pressure in common carotid artery in mm Hg; initial level of arterial blood pressure; manometer base line; marker denoting intravascular introduction of 20% NaCl solution; marker denoting insertion of syringe into catheter; time marker (5 seconds).

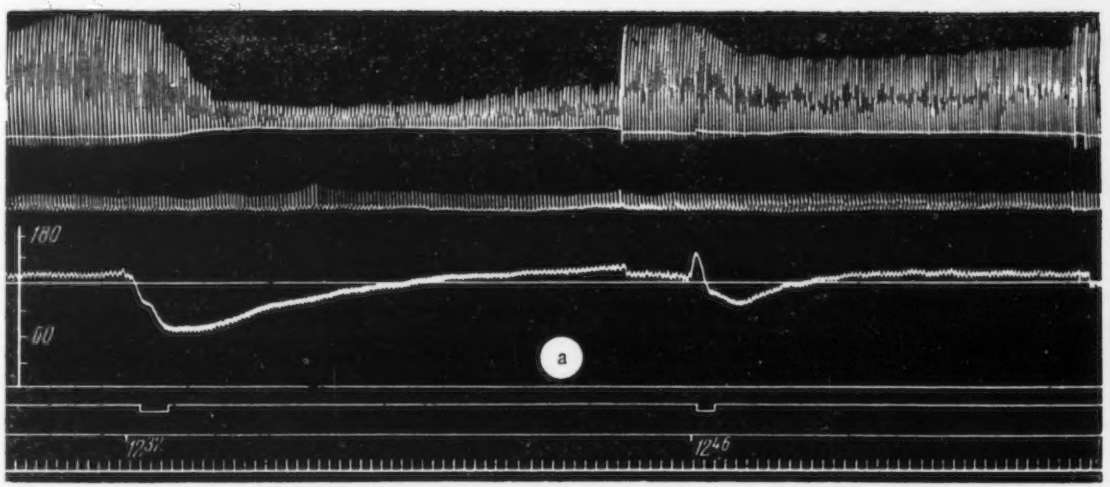

Fig. 2a. Changes in knee jerk reflex in response to introduction of 2 ml 20% NaCl solution into right auricle and thoracic aorta at T8 - T9 level (a) and 2 ml 20% NaCl solution into thoracic aorta at T8-T9 level and into right auricle (b) (cat under urethane anesthesia).

Records the same as in Fig. 1.

The results of the main series, consisting of 27 experiments, are shown in the Table.

Changes in the Knee Jerk Reflex on Intracardiac and Intra-Arterial Administration of Hypertonic (20%) NaCl Solution

Intensity of inhibition			Duration of inhibition		
Diminution of knee jerk (in % of initial amplitude)	Number of injections		Duration of inhibition of the knee jerk (in minutes)	Number of injections	
	Right auricle	Aorta		Right auricle	Aorta
76—100	25	9	Above 30	12	0
51—75	10	6	16—30	4	0
26—50	9	11	1—15	24	11
0—25	0	6	To 1	4	21
No change	6	18	No change	6	18
Total No. of injections	50	50	Total No. of injections	50	50

It is characteristic that in half of all the hypertonic saline injections into the right auricle the amplitude of the knee jerk decreased by more than ¾ of the initial magnitude, whereas on aortic administration this only occurred in 9 of the same number of injections. Moreover, intra-aortic administration was associated with absence of change in 3 times the number of cases as compared with intracardiac administration. The same is characteristic of the duration of reflex inhibition when the effects of hypertonic saline administration by the two routes are compared. Thus, intravenous injection led to inhibition lasting over 15 minutes in 16 of 50 cases, whereas intra-aortic administration did not elicit inhibition of similar duration in a single case; the effect of the latter was in most cases that of transient inhibition measurable in seconds.

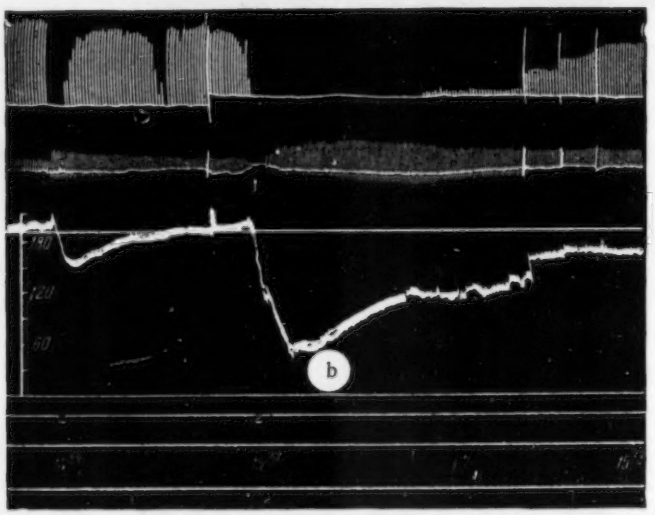

Fig. 2b. For legend see Fig. 2a.

Analysis of the table indicates that the inhibitory reaction is considerably more marked, both with respect to intensity and duration, in response to introduction of hypertonic NaCl solution into the right auricle than by the intra-aortal supply to the spinal cord (Figs. 1 and 2). The sequence of injections did not affect the character and magnitude of the response.

The experiments described show that changes elicited by intravenous injection of hypertonic NaCl solution are explained chiefly by reflex influences from cardiac and pulmonary receptors rather than by direct action of

the substance on the appropriate segments of the spinal cord. Professor V. S. Galkin had voiced suppositions of the existence of such influences on many occasions. It is not very clear yet which particular cardiac and pulmonary receptors are the most sensitive to this chemical agent. However, it is evident that when the substance by-passes the heart and lungs, passing into the arch of the aorta and then along the descending aorta to the spinal cord, it cannot elicit such marked changes. It must be taken into account that when the solution is given intra-aortically it reaches the spinal cord in higher concentration than when it is given by the intracardiac route, and especially by the intravenous route, but the reaction is nonetheless weaker. This fact further emphasizes the role of the reflex, interoceptive component in the mechanism of the described changes.

With respect to inhibition which is to some extent observed when the substance is supplied to the spinal cord by way of the aorta, it is necessary to take into account the existence of reflexogenic zones in the aorta itself [1, 6] on which the substance may act. This suggestion appears very probable because in special experiments in which the catheter was introduced into the abdominal aorta below the point from which the lumbar arteries arise (at the site where the aorta divides into the iliac arteries) inhibition of the knee jerk also took place in response to introduction of hypertonic NaCl solution.

The data presented indicate the need for considering the interoceptive component when analyzing the effects produced by intravascular administration of substances.

SUMMARY

The author compared the changes in the knee jerk occurring as a result of injection of hypertonic NaCl solution into the femoral vein, right auricle and various portions of aorta. Injection of hypertonic NaCl solution into the right auricle caused a more pronounced inhibition of the knee jerk than when it was injected into the aorta to supply the spinal cord. This suggested that the changes in the knee jerk caused by intravenous and intracardiac injection of the above substance was explained mainly by the reflex effect from the receptors of the lungs and heart, rather than by the direct action of this substance on the motor centers of the spinal cord.

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THE EFFECT OF POLARIZATION OF THE CORTEX AND SUBCORTICAL CENTERS ON THE MOTOR FUNCTION OF THE STOMACH AS SHOWN BY X-RAYS

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(Received June 7, 1957. Presented by Active Member AMN SSSR V. V. Parin)

The effect of cortical stimulation on the motor function of the stomach has been studied in short-term experiments by many investigators [16, 17, 1, 18, 20, 13, 12]. It was found that the areas of the brain which elicited the most marked changes in the motor function of the stomach were gyrus sigmoidea of the frontal region in its lateral, anterior and posterior parts and the adjacent areas of the cerebral cortex.

The effect of the hypothalamus on the motor function of the stomach was observed in short-term experiments by Beattie [14], Bodechtel and Kaufmann [15], Gellhorn [4], and Ström and Uvnäs [19]. I. A. Chereshnev [11] reports on the effect of stimulation of these formations on the evacuation function of the stomach as shown by x-rays; he used long-term experiments and noted slowing of the passage of barium from the stomach to the duodenum when the hypothalamus was subjected to chronic stimulation.

The roentgenographic method for the study of the motor-evacuation function of the stomach has been used fairly extensively. Many investigations [3, 7, 8, 9, 2] have demonstrated roentgenologically the special features of motor function of the normal stomach in dog. Its emptying, according to these authors, occurs in 2-4½ hours, the time of the appearance of contrast medium in the duodenum being 8-20 minutes.

The aim of the present work was the roentgenographic study of the effect of stimulation of the cortex and subcortical centers on the motor function of the stomach.

EXPERIMENTAL METHOD

In our roentgenographic studies of the changes in motor function of the stomach under the influence of polarization of the frontal cortex and anterior hypothalamus we used as contrast medium a thin gruel based on 3 g barium sulfate, 3 g semolina and 20 ml water per 1 kg body weight. The gruel was cooked for 5 minutes and was slightly salted. Some dogs ate it readily, others had to be spoon-fed. Prior to the experiment the dogs were fasting for 18-20 hours. At the beginning of each experiment the dogs were given the contrast medium and x-ray pictures were taken every 20 minutes (the experiment lasted 80 minutes). The dogs, tied down on a table, lay prone during the experiment. The cassette with film was placed under the abdomen. The normal motor function of the stomach was first determined in each dog, then (after determination of normal evacuation) stimulation of the cortex or subcortical centers or combination of the two was carried out at the beginning of each experiment.

Polarization of the cortex and hypothalamus was effected by means of in-dwelling electrodes in the frontal cortex in the projection area of the sigmoid convolution using A. B. Kogan's method and in the anterior hypothalamus by the G. Ia. Khvoles method.

In a series of experiments the frontal cortex was polarized by means of a plate electrode 3 × 3 cm in area which was applied to the shaved scalp. The results were similar to those obtained by the in-dwelling electrode method.

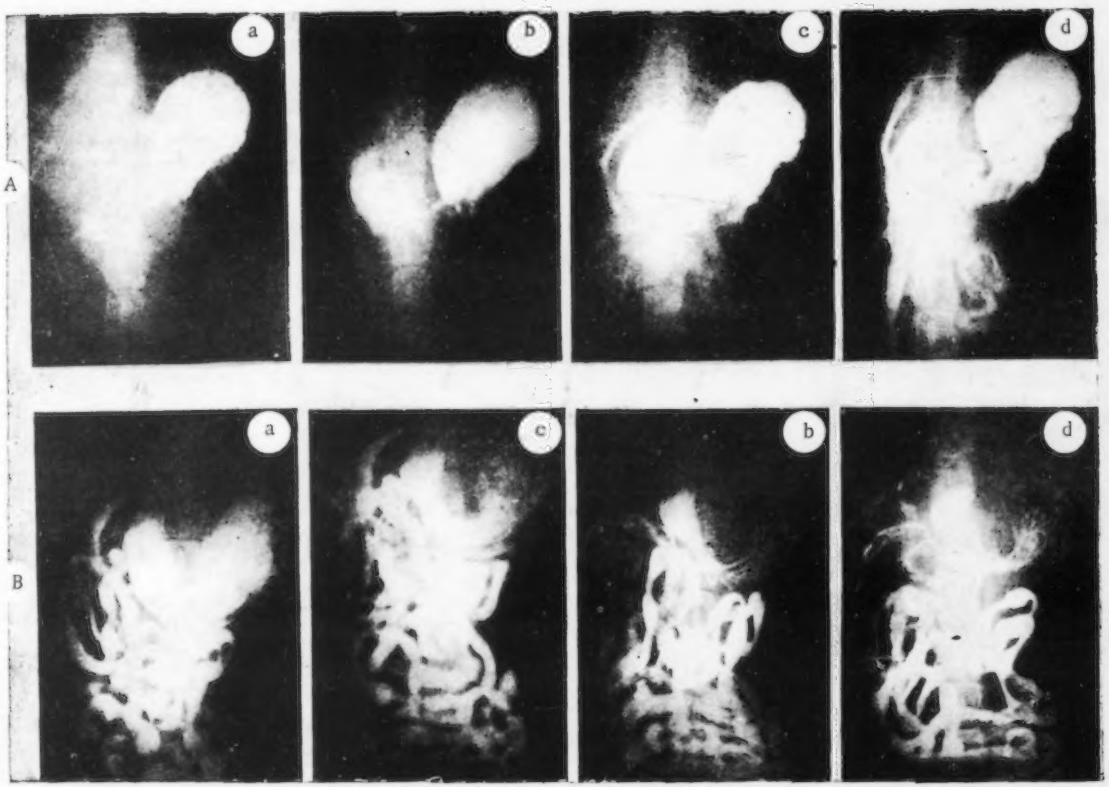

Fig. 1. Effect of galvanization of the frontal cortex with (A) cathode and (B) anode on the motor function of the stomach.

Roentgenograms of the stomach, dog Kashtanka, June 4, 1954 (upper row) and June 5, 1954 (lower row). Current strength 5 ma, duration of action 20 minutes; a) 20 minutes after stimulation; b) 40 minutes after stimulation; c) 60 minutes after stimulation; d) 80 minutes after stimulation.

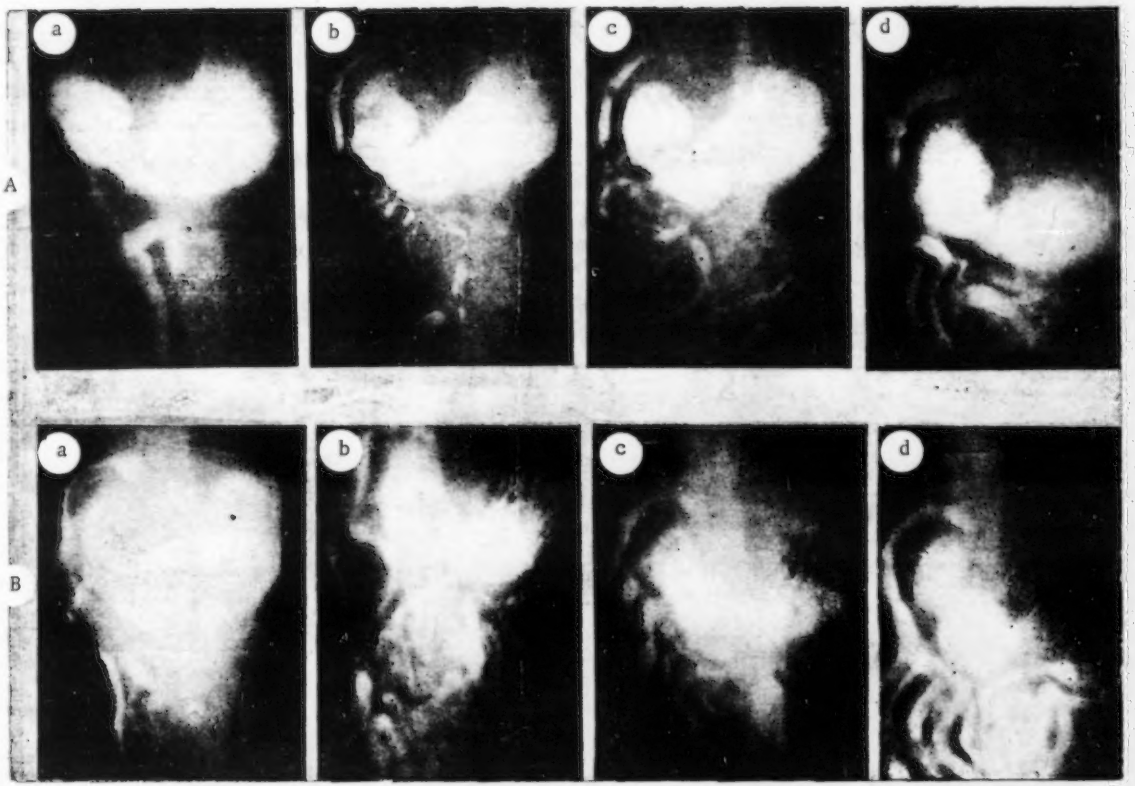

Fig. 2. Effect of galvanization of anterior hypothalamus with (A) cathode and (B) anode on the motor function of the stomach.

Roentgenograms of the stomach, dog Dzhul'bars, February 21, 1955 (upper row) and February 23, 1955 (lower row). Current strength 2 ma, duration of stimulation 10 minutes; a) 20 minutes after stimulation; b) 40 minutes after stimulation; c) 60 minutes after stimulation; d) 80 minutes after stimulation.

The indifferent electrode was placed on the dog's hind limb in the region of the outer surface of the hip.

Polarization was effected by galvanic current of 1 to 10 ma and by impulses of 0.5 - 2 ma over a period of 10-20 minutes.

The source of polarization current was a galvanizing apparatus fed by BAS-80 and GIF-constructed impulse generator.

A total of 100 experiments (400 roentgenograms) was performed on 8 dogs. In 62 experiments polarization of the cortex and subcortical centers was carried out, in the remainder the initial motor function of the stomach was determined. Emptying time as well as tone and peristalsis were studied. It is known that the shape of the stomach is in direct relation to the degree of its filling with contrast mass and to its tone. An atonic stomach has the appearance of a limp, distended bag; its volume decreases and its shape approaches the oval or spherical as the tone increases [6, 9]. During the time of an experiment (80 minutes) the contrast medium managed to pass into the intestine emptying almost the whole stomach and being retained only at the pylorus and in the duodenal bulb.

EXPERIMENTAL RESULTS

Galvanization of the frontal cortex with a cathode caused sharp depression of the motor function of the stomach. After 80 minutes the contrast medium filled the whole stomach with the exception of the upper parts of the antrum, appearing in separate portions in the small intestine. In many experiments marked demarcation of the pyloric part of the stomach was noted, caused by spasm of the prepyloric sphincter. The outline of the pylorus was not detected since it was hardly filled; its shape was elongated, indicating a sharp rise in its tone; later it was filled with contrast medium (Fig. 1, A). In a number of experiments with 10-minute galvanization the cardial-fundal part of the stomach was atonic.

Similar but even more marked inhibitory effect on the motor function of the stomach was caused by polarization of the frontal area with a cathode and impulse current at the rate of 30 to 300 impulses per second. During the 80 minutes' observation the outline of the stomach was markedly atonic in character. The pyloric tone was increased.

The slowing of passage of contrast medium from the stomach observed during cathodic polarization of the frontal cortex is thus determined by the increased tone of the pyloric sphincter and sometimes by atonia of the fundal-cardial part of the stomach.

Anodic glavanization of the cerebral cortex produced in most experiments acceleration of evacuation; less often it produced no effect. Accelerated evacuation of the stomach under the influence of anodic galvanization is illustrated by gastric roentgenograms obtained from the dog Kashtanka on June 5, 1954 (Fig. 1, B).

The current strength was 5 ma, duration of polarization 20 minutes. Evacuation function of the stomach was clearly evident on the 20th minute. Its volume decreased, and its surface showed constrictions. These are manifestations of gastric peristalsis. (The colon is filled with contrast medium which remained after cathodic stimulation of the cortex performed on June 4, 1954.).

60 minutes after administration of the contrast medium almost complete emptying of the stomach could be observed; in this experiment the spastic shape of the stomach is characteristic (Fig. 1, B).

Polarization of the frontal cortex using an anode and current impulses of frequencies equal to 30, 100 and 300 impulses per second produced in the first 40 minutes a general increase of tone in the smooth musculature of the fundal-cardial part, spasm of the prepyloric sphincter and a sharp demarcation of the body of the stomach from the pylorus (first phase). The roentgenographic appearance of the stomach was of the shrunken spherical type with sharp delineation of it from the hardly filled pylorus. Later (second phase) evacuation occurred normally and after 80 minutes' observation the stomach acquired a very elongated shape and was almost half filled with contrast medium.

Cathodic polarization of the anterior hypothalamus with impulse and galvanic current at frequencies of 30, 100 and 300 impulses per second gave inhibition of evacuation owing to gastric hypotonia and spasm of the pyloric sphincter.

The roentgenogram of the dog Dzhul'bars' stomach (Fig. 2, A) shows gastric hypotonia and pyloric sphincter spasm over a period of 80 minutes. The contrast medium fills the stomach and is present in separate portions in the small intestine.

Anodic galvanization of the anterior hypothalamus was always accompanied by timely emptying of the stomach after a preliminary (first) inhibitory phase (20-30 minutes) which was characterized by relaxation of the cardial-fundal part of the stomach.

The roentgenograms of Dzhul'bars' stomach (Fig. 2, B) after anodic stimulation of the anterior hypothalamus with current strength of 2 ma for 10 minutes show the stomach to be distended, bag-like and filled with contrast medium (first phase). After 40 minutes the contrast medium appeared in the small intestine, emptying the cardial--fundal part of the stomach; there was increase in gastric tone (second phase). A constriction appeared in the region of the prepyloric sphincter. After 80 minutes the rate of evacuation approached the initial.

The second phase of motor activity of the stomach during galvanization of the anterior hypothalamus is thus characterized by enhancement of evacuation.

Anodic polarization of the anterior hypothalamus at impulse rates of 30, 100 and 300 per second inhibited the motor evacuation of the stomach as the result of prepyloric sphincter spasm and gastric atonia which appeared 40 minutes after stimulation.

Polarization of the brain by ascending or descending galvanic or impulse current of any frequency (30, 100, 300 per second) inhibited the passage of contrast medium from the stomach to the duodenum. This inhibition occurred because of pyloric sphincter spasm and general lowering of gastric tone when polarization was effected by ascending current and only because of pyloric spasm when polarization was achieved by descending current, when gastric tone was increased and peristalsis enhanced.

The inhibitory action of descending impulse current was less marked.

The predominant effect of anelectrotonus during polarization of the cortex and subcortical centers on the motor function of the stomach was activation, that of catelectotonus was inhibition.

Our investigations confirm the data concerning the significance of the frontal cortex and the anterior hypothalamus with respect to the motor function of the stomach.

SUMMARY

Experimental data concerning the effect of stimulation of cortical and subcortical centers on stomach evacuation are presented. Cathodic stimulation of the cortex or hypothalamus depresses the evacuation function of the stomach. The anodic effect is biphasic and reveals the inductive relationship between the cortical and subcortical centers.

Simultaneous stimulation by galvanic descending current (anode on the cortex and cathode on hypothalamus), or by ascending current (cathode on the cortex and anode on hypothalamus) depresses evacuation of the stomach. Stimulation by impulse current results in more rapid evacuation.

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INCREASED WORK CAPACITY OF MUSCLE ON STIMULATION THROUGH THE NERVE AND THE CAUSES OF THIS PHENOMENON

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It is commonly known that gradually increasing expenditure of substances and accumulation of decomposition products such as inorganic phosphorus and lactic acid occur in the neuromuscular preparation during work; this is accompanied by a decline of work capacity. These phenomena are also noted in experiments on muscle in situ with preservation of blood supply and innervation [3, 5, 17, 18, 20].

In studying questions connected with the mechanism of neuromuscular transmission of excitation in situ [2, 7, 8, 9] we succeeded in observing such tetanic contraction of muscle under certain conditions of indirect stimulation during which the muscle tissue content of phosphorus not only did not increase in relation to the initial resting state but even diminished, and there was delay in the development of fatigue. A description of the condition requisite for the appearance of this phenomenon and an analysis of its cause are given below.

EXPERIMENTAL METHOD

Experiments were performed on white rats under ether anesthesia. The cut tendon of the gastrocnemius muscle in situ was connected to a stylus. Square-wave and saw-tooth impulses generated by an electronic stimulator GRAKh-1 were used as stimuli.

One of the symmetric muscles was stimulated through buried platinum electrodes via the nerve, the other directly through platinum needles inserted into the proximal and distal ends of the muscle.

After the experiment both the muscles were excised at the same time and frozen with liquid air. Inorganic phosphorus and lactic acid were determined in the powder obtained by triturating the muscle in a mortar with liquid air, the former by the microcolorimetric method following reduction of the phosphorus-molybdenum complex with stannous chloride in butanol extract of the trichloroacetic acid extract [21], the latter with the help of p-hydroxydiphenyl [12].

EXPERIMENTAL RESULTS

It is known that when nerve is stimulated by frequency or strength of stimulation in excess of a certain value, the muscle relaxes instead of contracting, a phenomenon referred to as pessimum [6, 8, 13].

However, in the majority of pessimal states described in the literature the action of the stimulus was not confined to the area of neuromuscular transmission but affected the muscle also, owing to which the latter showed signs of exhaustion with complete pessimal relaxation and absence of any external work [2, 8, 10, 11]. We discovered that on stimulation with square-wave and saw-tooth pulses of long duration (10 milliseconds) at frequencies of around 53-55 cps and moderate strength, complete pessimal relaxation of muscle was observed only on account of block in the intermediate link, without involving the muscle fibers. We designated this state as "true pessimum" [2, 8].

When the stimulus strength is gradually increased beginning with subthreshold, muscle being stimulated indirectly responds first, followed by response from the directly stimulated muscle also. The level of tetanus observed in the latter is lower than that given by the indirectly stimulated muscle when the strength of stimulation applied is the same. When stimulus strength is increased further the height of optimal contractions of both muscles becomes equalized.

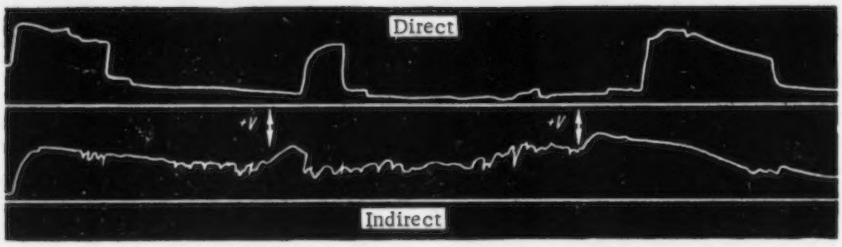

Fig. 1. Examples of investigated form of prolonged tetanus with direct and indirect stimulation (explanations in text).

When stimuli with parameter values close to the pessimal (duration 10 milliseconds, frequency 50-52 cps) but still giving optimal response are used, indirect stimulation (Fig. 1) leads to tetanus which does not pass into relaxation during continuous action but assumes an undulating course, sometimes even without appreciable lowering of the summated height of contractions. Direct stimulation under similar conditions leads to rapid relaxation with no or slight secondary rises, the most frequently observed effect being that of a sharp decline to the base line. This relaxation of the muscle to zero level during continuing direct stimulation attests to a steeper rise in stimulation threshold than in the case of similarly prolonged indirect stimulation.

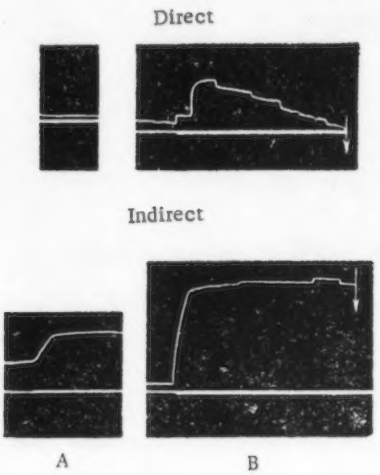

Fig. 2. Examples of investigated forms of brief tetanus during direct and indirect stimulation (explanations in text).

During direct stimulation of muscle a small increase in stimulus strength (arrows in Fig. 1) usually elicits a sharp increase in the height of contractions, following a considerable latent period, which is replaced by a sharp fall. Indirect stimulation under these conditions leads to a much more stable increment in the height of contractions.

We investigated the accumulation of inorganic phosphorus during the described simultaneous direct and indirect stimulation of symmetrical muscles in the same animal. Two stimulus strengths were investigated: one which elicited no response from the directly stimulated muscle and submaximal tetanus from the indirectly stimulated muscle (sections of myograms A in Fig. 2) and the other which elicited submaximal tetanus from the directly stimulated muscle and a higher tetanus from the indirectly stimulated muscle (myogram examples B in Fig. 2). In both cases the work performed in lifting the load was apparently greater in the case of the indirectly stimulated muscle. This would appear to require greater metabolic turnover, with greater accumulation of inorganic phosphorus resulting from the decomposition of its organic forms as compared with the directly stimulated muscle. Experimental findings, however, revealed just the opposite. During brief work (about 1 minute) the indirectly stimulated muscle invariably showed less inorganic phosphorus than the directly stimulated one.

These results are presented in the table which shows the data of several typical experiments. It demonstrates that in all cases the changes in inorganic phosphorus content compared with the mean data for the resting state are opposite for directly and indirectly stimulated muscles. This effect is also apparent on comparison with mean data obtained on different animals, but is particularly clear in experiments on symmetrical muscles of the same

animal. It would seem that under such conditions of stimulation we observe forms of activity approaching the physiologic. This is also supported by the absence of accumulation of lactic acid. The absence of differences in lactic acid content of directly and indirectly stimulated muscles is explained by the lower sensitivity of this index to changes in the functional state.

The described effect of opposite shifts in inorganic phosphorus content can only be observed during brief activity of both muscles.

More prolonged stimulation (5-7 minutes) (Fig. 1) is shown by biochemical data to be associated with much more profound shifts in the case of both the directly and indirectly stimulated muscles. Under these conditions it is no longer possible to detect any consistency in the relations of the extent of change during direct and indirect stimulation. Myograms typical for this situation (Fig. 1) show that performance of such prolonged work is associated with a rise in threshold. Therefore, in order to maintain contraction at a definite level, it was necessary to increase very slightly the amplitude of stimulation several times (arrows on the myograms).

Inorganic Phosphorus and Lactic Acid Content in Directly and Indirectly Stimulated Symmetrical Muscles During Different Forms of Activity.

Each pair of values across represents results of one experiment on symmetrical muscles
The average inorganic phosphorus content at rest is 17.5 mg%, of lactic acid 144 mg%

Inorganic phosphorus				Lactic acid			
In mg % calculated on net tissue weight							
Tetanus				Tetanus			
Brief		Prolonged		Brief		Prolonged	
Stimulation				Stimulation			
direct	indirect	direct	indirect	direct	indirect	direct	indirect
19.0	15.0	45.0	41.0	130	130	250	248
17.5*	14,5	47.0	50.0	143	140	270	250
17.5	13,0	46.0	50.0	145	148	260	320
18.0*	16,5	68.0	65.0	138	140	380	365

*Examples of stimulation with the weaker stimulus strength of those investigated, when direct stimulation elicited no muscular contraction.

The data given in the table show that during prolonged stimulation the inorganic phosphorus and lactic acid content of muscle increases whether stimulation is direct or indirect.

No consistent distinction in terms of biochemical indices can be made between directly and indirectly stimulated muscles when they are briefly stimulated with current strengths which elicit maximal response from both muscles.

The described favorable shift in the state of the muscle during activity elicited by indirect stimulation cannot be explained by internal stimulation of metabolism by decomposition products arising during moderate activity [18], since analogous phenomena should have been noted during submaximal tetanic contraction of directly stimulated muscle also.

Since no such phenomena are observed, the cause of the effect under consideration must be sought in some other influences arising in muscle stimulated through the nerve.

We have already mentioned that for the given type of stimuli of long duration it is sufficient to increase the frequency very slightly (by 3-5 cps) in order to obtain pessimal relaxation instead of optimal contraction. This pessimum, unlike other forms, represents extremely selective blocking of neuromuscular transmission owing to which muscle tissue is completely untouched by the action of the stimulus ("true pessimum"). It is known that

tissue altered by a stimulus and bordering on unchanged tissue elicits in the latter, perielectrotonically, a state opposite to its own [1, 19, 20]. Therefore the occurrence of complete blocking of transmission with signs of the second phase of parabiotic process in neuromuscular transmission is responsible for the fact that the state of relaxing muscle fibers in "true pessimum" does not simply return to the initial resting level but improves on it (in "true pessimum" muscle tissue shows lowering of inorganic phosphorus content and lowering of thresholds to stimulation as compared to the resting state) [2, 3, 10, 12, 13].

Despite the sharp differences of external manifestation of pessimum and optimum reactions, the improvement in the condition of the muscle fibers in both cases is evidently due to the same mechanism of neuromuscular transmission block.

This becomes apparent on investigation of the muscular response which is intermediate between the optimal and pessimal [2, 3, 10, 12, 13]. Such a reaction consists of rhythmic alternation of muscular contraction and relaxation during continuous stimulation maintained for an indefinitely long period of time, with no signs of fatigue and even with slow but definite improvement on the initial condition. The mechanism responsible for the development of favorable changes in the muscle in this case too is the development of block in neuromuscular transmission. This block, during continuous stimulation, can appear and disappear periodically only when the frequency of stimulation is lowered compared to the pessimal [12]. It would appear that on further lowering of stimulation frequency the periodicity of appearance and disappearance of this block occurs with an even higher rhythm, which leads to a tetanic response. The similarity between the described tetanic contraction and rhythmic activity is confirmed by the coincidence of a number of conditions under which they become manifest, as well as by the fact that this form of tetanus passes into wave-like contraction approaching rhythmic contraction when stimulation is prolonged.

It is relevant to mention the work of A. I. Rappoport [13] and I. M. Bocharnikova in connection with the fact that the described increased capacity for work observed in muscle stimulated by way of the nerve disagrees with the widely held view that muscle is more vulnerable under such conditions of stimulation; these authors have shown that the latter concept is not valid for physiologic conditions. When experiments are staged under conditions closer to the natural, with constant washing away of products of activity toxic to the neuromuscular junction, it is seen that it is the nerve-stimulated muscle which preserves excitability for longer periods. There are grounds, therefore, for considering that the possibility of increasing the working capacity of muscle by its indirect stimulation, as discovered in our experiments, also occurs during natural physiologic activity.

SUMMARY

Indirect stimulation of the rat neuromuscular preparation in situ by square-wave and saw-tooth electric current impulses with the frequency of 50-52 cycles/second, 10 msec duration causing submaximal or initial maximal responses gives rise to a peculiar tetanic contraction.

The usual increase of lactic acid in muscle tissue is absent in this contraction; the quantity of inorganic phosphorus in it even decreases compared with the value during the initial condition of rest, while the development of fatigue is retarded as compared to the directly stimulated muscle. The peculiarities of tetanus are connected with increased working capacity of the muscle as a result of the form of activity described above.

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

ESTIMATION OF THE DOSE OF ANTIGEN REQUIRED TO PRODUCE ANTIBODIES UPON INJECTION INTO THE CAROTID SINUS

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In 1945 we showed [1] that the injection of an antigen into the isolated carotid sinus leads to the formation of agglutinins. This observation was confirmed by other workers and by our own research. At the same time investigations have been reported from which it follows that antibody formation takes place only in cases when the antigen is absorbed [3]. According to other workers, in general it is impossible to isolate the carotid sinus and the formation of antibodies in response to injection of antigen in the isolated carotid sinus depends on the absorption of the antigen [4].

Without stopping to criticize the investigations and in view of the fact that these workers were unable to isolate the carotid sinus, we would like to point out that success in this operation depends on the method of isolation used and on the skill of the experimenter. Experiments in our laboratory have shown that isolation of the carotid sinus is perfectly possible and easily performed [2].

In this paper we try to reach a decision about the reflex production of antibodies by using a different approach. If the authors mentioned above are right in assuming that antibody production takes place only through resorption of antigen injected into the carotid sinus, then two questions need to be answered: 1) what is the minimum quantity of antigen which, injected intravenously, will produce antibodies and 2) what quantity of antigen injected into the isolated carotid sinus will have the same effect. From a comparison of these two values we shall be able to decide what part is played by resorption in the development of this process and does it exclude a reflex mechanism even if resorption does take place when the experiments are performed in this way.

EXPERIMENTAL METHOD

The experiments were carried out on dogs. Altogether 36 experiments were performed on 36 dogs. The weight of the dogs varied from 8 to 12 kg. In some dogs the carotid sinus was isolated by a method previously described by us. A vaccine of killed dysentery bacilli, containing a varying number of organisms, was injected into the isolated carotid sinus. After the vaccine had been in contact with the receptors of the carotid sinus for five minutes additional ligatures were applied parallel to the first ligatures, the intervening ligatures were divided and the carotid sinus, filled with vaccine, was removed. Before injection of the vaccine into the sinus blood was taken and the titer of antibodies to dysentery and paratyphoid bacilli in this serum was determined. Determination of the agglutinin titer to the same organisms was repeated after 7 days. We established the smallest dose capable of causing the formation of agglutinins when injected into the sinus and then investigated the possibility of formation of agglutinins by intravenous injection of the same dose or of a part of it.

EXPERIMENTAL RESULTS

Blood was taken from 6 dogs for determination of the agglutination titer of their serum. Next the carotid sinus was isolated in each dog. Into the isolated sinus was injected 0.25 ml of a vaccine containing 100 million dysentery bacilli in 1 ml. The isolated sinus, containing the vaccine, was removed after 5 minutes. Blood was

taken from all the dogs 7 days later and the agglutinin titer of the serum determined (Table 1).

TABLE 1

Changes in the Agglutinin Titer After Injection of Microorganisms into the Isolated Carotid Sinus

№	Titer			
	initial		after 7 days	
	antigen			
	dysentery bacillus	paratyphoid B bacillus	dysentery bacillus	paratyphoid B bacillus
	25 million organisms			
1	1 : 20+	1 : 20+	1 : 320+	1 : 20+
2	1 : 20—	1 : 20	1 : 160+	1 : 20+
3	1 : 20	1 : 40	1 : 160+	1 : 40+
4	1 : 40	1 : 20	1 : 320	1 : 80
5	1 : 20—	1 : 80	1 : 320	1 : 80
6	1 : 20	1 : 20	1 : 160	1 : 40
	100,000 organisms			
1	1 : 20	1 : 20	1 : 160	1 : 40
2	1 : 10	1 : 20	1 : 160	1 : 20
3	1 : 80	1 : 40	1 : 320	1 : 160
4	1 : 40	1 : 20	1 : 640	1 : 20
5	1 : 20	1 : 20	1 : 160	1 : 40
6	1 : 40	1 : 20	1 : 80	1 : 20
	25,000 organisms			
1	1 : 20	1 : 20+	1 : 40	1 : 20
2	1 : 40	1 : 20	1 : 640	1 : 20
3	1 : 20	1 : 20	1 : 40	1 : 20
4	1 : 20	1 : 20	1 : 160	1 : 20
5	1 : 40	1 : 20	1 : 320	1 : 20
6	1 : 20	1 : 40	1 : 80	1 : 40
	2 500 organisms			
1	1 : 20	1 : 20	1 : 20	1 : 20
2	1 : 20	1 : 20	1 : 20	1 : 20
3	1 : 40	1 : 20	1 : 20	1 : 20
4	1 : 20	1 : 20	1 : 20	1 : 20
5	1 : 40	1 : 40	1 : 40	1 : 20
6	1 : 80	1 : 20	1 : 80	1 : 20

The titer of agglutinins to the dysentery bacillus in the serum of the experimental dogs before the injection of vaccine varied between limits of 1 : 20 to 1 : 40 and that to the paratyphoid B bacillus between limits of 1 : 20 to 1 : 80.

On the 7th day after the injection of 25 million dysentery bacilli into the sinus the agglutinin titer to these organisms rose to within limits of 1 : 160 to 1 : 320. The titer of agglutinins to the paratyphoid B bacillus in the majority of cases was unchanged and only in one case was it increased from dilutions of 1 : 20 to 1 : 80.

The results of this series of experiments show that injection of 25 million microorganisms into the isolated carotid sinus causes agglutinin formation in dogs.

In another 6 dogs the same procedure was followed as in the foregoing group but an injection of 100,000 dysentery bacilli in a volume of 0.25 ml was given into the carotid sinus (see Table 1). The experiments showed that this dose of antigen also causes antibody formation. The titer of agglutinins to the dysentery bacillus varied before immunization between limits of 1 : 20 to 1 : 80 and after immunization between limits of 1 : 160 to 1 : 640; that to the paratyphoid bacillus was unchanged.

TABLE 2

Agglutinin Titer in Dogs After Intravenous Injection of Antigen

№	Titer			
	initial		after 7 days	
	antigen			
	dysentery bacillus	paratyphoid B bacillus	dysentery bacillus	paratyphoid B bacillus
	2500 organisms			
1	1 : 80	1 : 80	1 : 80	1 : 80
2	1 : 20	1 : 20	1 : 20	1 : 20
3	1 : 20	1 : 80	1 : 20	1 : 40
4	1 : 40	1 : 20	1 : 40	1 : 40
5	1 : 20	1 : 20	1 : 20	1 : 20
6	1 : 20	1 : 20	1 : 20	1 : 40
	25,000 organisms			
1	1 : 20	1 : 20	1 : 20	1 : 20
2	1 : 20	1 : 20	1 : 20	1 : 40
3	1 : 40	1 : 40	1 : 40	1 : 20
4	1 : 20	1 : 40	1 : 20	1 : 20
5	1 : 80	1 : 20	1 : 80	1 : 20
6	1 : 20	1 : 40	1 : 40	1 : 40

In the next group of experiments 25,000 dysentery bacilli were injected into the isolated carotid sinus of 6 dogs under the same conditions. This number of dysentery bacilli, injected into the isolated carotid sinus in a volume of 0.25 ml of fluid, causes a reliable increase in the agglutinin titer in the majority of cases and in a smaller number of cases an unreliable increase.

In the fourth group a dose of 2500 organisms was used. The injection of such a dose into the isolated carotid sinus caused no change in the agglutinin titer. The results of these experiments are shown in Table 1.

These groups of experiments show that the threshold dose of dysentery bacilli capable of causing agglutinin formation on injection into the isolated carotid sinus is 25,000 organisms. In the majority of dogs this dose produces a reliable increase in the agglutinin titer.

Having established the threshold dose and admitting a maximum possible resorption of 10% (10 times higher than that demonstrated by I. A. Oivin and Iu. V. Sergeev [4]), we attempted to find out whether this dose causes an increase in the agglutinin titer when injected into the blood stream. For this purpose 6 dogs were injected with 0.25 ml of physiological saline containing 2500 dysentery bacilli and after 7 days their agglutinin titer was determined. The agglutinin titers to both paratyphoid and dysentery bacilli were found to be unchanged (Table 2). Since injections of 2500 organisms into dogs had not altered their agglutinin titer we increased this dose to 25,000 organisms. Intravenous injection of 25,000 dysentery bacilli likewise caused no change in the agglutinin titer, as can be seen in Table 2.

The results obtained demonstrate that an injection of 25,000 dysentery bacilli into the isolated carotid sinus

is capable of causing agglutinin formation in dogs and that this does not occur if the same number of dysentery bacilli is injected intravenously. These results dispel all doubt that a vaccine injected into the isolated carotid sinus acts primarily on its receptors and causes antibody formation in a reflex manner. If resorption takes place as a result of unskillful isolation of the carotid sinus, this may account for the denial of a reflex mechanism of antibody formation.

SUMMARY

The minimal quantity of microbic bodies capable of causing antibody production when in contact with receptors of isolated carotid sinus was determined. It was demonstrated that this antigenic dose is not connected with increase of the titer of specific agglutinins, when injected intravenously to dogs of the same weight. This is an additional confirmation of the possibility of the reflex mechanism of antibody production.

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DISTINCTIVE FEATURES OF THE ACTION OF HYPNOTICS AFTER NOCICEPTIVE STIMULATION OF ANIMALS IN A STATE OF HYPOTHYROIDISM*

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The individual reactivity of the body has recently become the focus of attention of research workers. Its study became possible when experimenters changed over from the investigation of the functions of isolated organs and systems to that of the coordinated activity of the body as a whole.

The modern view of the problem of individual reactivity holds that it is based on a series of factors which ultimately result in differences in the functional state of the nervous system. The importance of individual reactivity clearly arises during investigation of the action of various pharmacological drugs on animals and man [10, 14, 16, 18, 19]. One of the factors changing the functional state of the nervous system and, consequently, the reactivity of the body is pain (nociceptive stimulation).

The influence of nociceptive stimulation on the action of drugs has been investigated in various forms of experiments by many workers: P. A. Ivanov [13], V. V. Zakusov [11, 12], S. G. Kuznetsova [17], S. M. Dionesov [5, 6], S. M. Dionesov and A. G. Usov [8, 9], B. G. Volynskii [4] and others. The experiments of these authors demonstrated that different drugs have an action on the body which differs in intensity (and sometimes in a qualitative respect also), if before the administration and sometimes immediately afterward the animal is subjected to brief nociceptive stimulation. These experiments showed that the action of drugs consequently depends on the individual reactivity of the body.

During a study of the distinctive features of the action of hypnotics on "normal" animals after nociceptive stimulation, creating a temporary functional disorder of the animal, we thought that it would be useful to find out how the same drugs would act after nociceptive stimulation in a case where the functional state of the animal was alreday altered. As a factor altering the functional condition of the nervous system for a relatively long period of time, and hence the reactivity of the body too, we selected experimental hypothyroidism in which, as has been shown by several authors [1-3, 15 and others], the tonus of the nervous system is diminished.

EXPERIMENTAL METHOD

The experiments were carried out on adult male white mice. A hypothyroid state was induced in them by intragastric injection (for 3 weeks) of a suspension of methylthiouracil in a dose of 5 mg of the drug per day per mouse by means of a tube. For the investigations we selected chloral hydrate and sodium amytal, whose action after nociceptive stimulation we had studied previously [6, 8, 9]. The hypnotic drugs were injected subcutaneously into the mice: chloral hydrate in 3% solution in a dose of 0.3 mg/g, sodium amytal in 0.004% solution in a dose of 0.06 mg/g. The indicator of hypnotic action was the "lateral position." Nociceptive stimulation was applied to part of the animals 5 minutes before the injection of the hypnotics, and lasted for 30 seconds. For stimulation an apparatus constructed by V. V. Levoshin was used, allowing strong compression of the tail of the mice, at the

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base, to be carried out in accurately graded doses. Altogether 1031 experiments were performed.

EXPERIMENTAL RESULTS

Experiments with chloral hydrate. In a previous paper [7] we found a sharp reduction in the hypnotic action of chloral hydrate in mice receiving methylthiouracil for a period of 3 weeks. If in control experiments the "lateral position" was observed in 34% of mice, on the 11th day after the start of administration of methylthiouracil this position was observed in 25% and on the 21st - 22nd day, in only 4% of the animals. Thus chloral hydrate acted considerably less strongly on the hypothyroid animals than on the "normal" animals, and its effect gradually weakened as the duration lengthened (of administration of the drug) blocking the function of the thyroid gland.

We obtained different results after injection of chloral hydrate to hypothyroid animals after nociceptive stimulation. In these experiments the hypnotic action of chloral hydrate was considerably more pronounced: on the 10th-14th day after the start of methylthiouracil administration the "lateral position" appeared in 43% of the animals and on the 21st-22nd day, in 37% of the animals, thereby reaching the level of hypnotic action of chloral hydrate on "normal" intact animals (Table 1).

TABLE 1

The "Lateral Position" After Injection of "Normal" and Hypothyroid Mice with Chloral Hydrate in Control Experiments and After Nociceptive Stimulation

Control experiments on "normal" mice			Experiments on hypothyroid mice						Day after beginning of administration of methylthiouracil
			Control experiments			Experiments with nociceptive stimulation			
total number of mice	"lateral position"		total number of mice	"lateral position"		total number of mice	"lateral position"		
	no.	%		no.	%		no.	%	
258	95	37	100	25	25	64	28	43	10—14
			100	4	4	62	23	37	21—22

Experiments with sodium amytal. The hypnotic action of sodium amytal on hypothyroid animals, as shown by our earlier experiments [7], was weaker than in the "normal" animals. In the control experiments, for instance, the "lateral position" was observed in "normal" animals in 51% of cases and in hypothyroid animals on the 21st day after the beginning of methylthiouracil administration in only 9% of cases. After nociceptive stimulation of hypothyroid animals the "lateral position" was observed on the 10th-11th day from the start of methylthiouracil administration in 8% of cases and on the 22nd day – in only 4% of cases. Consequently nociceptive stimulation in practice hardly changed the incidence of appearance of the "lateral position" in the hypothyroid animals. (Table 2).

Neither in the experiments with chloral hydrate nor in those with sodium amytal could we observe any regular feature in the rate of appearance of the "lateral position" and its duration.

In previous investigations [8, 9] we ascertained that after strong nociceptive stimulation the hypnotic action of chloral hydrate is enhanced and that of sodium amytal is diminished. In investigations carried out on hypothyroid mice [7] we found a significant weakening, in comparison with normal, of the hypnotic action of both chloral hydrate and sodium amytal which, in our opinion, is explained by weakening of the process of inhibition as well as that of excitation.

In the present investigation it was discovered that after nociceptive stimulation of hypothyroid animals the intensity of the hypnotic action of both test drugs was affected in the same direction as in "normal" animals; the degree of the changes in intensity of action of these drugs was, however, expressed otherwise. In the control experiments on hypothyroid animals, for instance, the "lateral position" was observed after injection of chloral hydrate in only 4% of animals, but after nociceptive stimulation – 37% of animals. Such a sharp increase in the in-

tensity of the hypnotic action of chloral hydrate is probably explained by the fact that on a background of a sharply lowered tonus of the nervous system a powerful factor such as nociceptive stimulation is capable of causing a considerably larger change in reactivity than when the tonus of the nervous system in the intact animals is "normal", and in its turn this determines the intensity of action of the hypnotic drug. In consequence of this, the increase in the hypnotic action of chloral hydrate observed in our experiments was so great that the "lateral position" was observed as frequently as in the control experiments on "normal" animals.

TABLE 2

The "Lateral Position" After Injection of "Normal" and Hypothyroid Mice with Sodium Amytal in Control Experiments and After Nociceptive Stimulation

Control experiments on "normal" mice			Experiments on hypothyroid mice						Day after beginning of administration of methylthiouracil
			Control experiments			Experiments with nociceptive stimulation			
total number of mice	"lateral position"		total number of mice	"lateral position"		total number of mice	"lateral position"		
	no.	%		no.	%		no.	%	
292	149	51	—	—	—	51	4	8	10—11
			55	5	9	49	2	4	21—22

In the control experiments on hypothyroid animals sodium amytal elicited the "lateral position" in 9% of the animals, but in the experiments with nociceptive stimulation – in 4% of animals. In this case perceptible changes in the hypnotic action of sodium amytal were practically absent; this was probably due to the fact that in the control experiments on hypothyroid animals the intensity of the hypnotic action of sodium amytal was slight, and so significant changes in its intensity could not be detected even if they occurred. Nevertheless, it must be pointed out that after nociceptive stimulation a tendency was observed for the hypnotic action of sodium amytal to be reduced, i.e., changes in the hypnotic action were brought about in the same direction as in the "normal" animals.

The results which are given in this paper convince us that the individual reactivity of animals under the influence of nociceptive stimulation is modified in the same direction when this stimulation is applied to an animal with "normal" tonus of the nervous system or to one with diminished tonus (during experimental hypothyroidism).

SUMMARY

The sleep-inducing effect of chloral hydrate (0.3 mg/g body weight) and sodium amytal (0.06 mg per g) was studied on white mice with hypothyroidism. The latter was attained by daily (during 3 weeks) administration of methylthiouracil (5 mg per mice) by a stomach tube. Pronounced nociceptive stimulation preceded the administration of sleep-inducing drugs in certain experiments. Sleep-inducing substances caused much less effect on animals with hypothyroidism than on normal animals. After nociceptive stimulation the sleep-inducing effect of chloral hydrate on animals with hypothyroidism was much less than that in normal animals. After nociceptive stimulation the sleep inducing effect of chloral hydrate increased in animals with hypothyroidism more intensely than in in normal animals, while the effect of sodium amytal had a tendency to decrease.

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THE EFFECT OF CENTRIPETAL ACCELERATION ON THE CONTENT OF ACETYLCHOLINE, ADRENALIN, ADRENALIN-LIKE SUBSTANCES, POTASSIUM AND SODIUM IN THE BLOOD OF ANIMALS

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In spite of the large number of papers dealing with the effect of acceleration on the body, the mechanisms of regulation of function during the action of acceleration remains far from clear. Accordingly, the aim of our investigation was to study certain aspects of the neuro-humoral mechanisms of regulation.

EXPERIMENTAL METHOD

The work was carried out on white rats (over 300) and five dogs. The animals were exposed to centripetal acceleration of magnitude 4-10 g for varying exposures and in varying directions.* For the rats we used a centrifuge of radius 1.25 m and for the dogs one with radius 3.6 m. Estimations were made of the acetylcholine, adrenalin-like substances, and potassium and sodium ion contents of the blood of the animals. Blood was taken from the rats by decapitation, and from the dogs – from the femoral vein.

The adrenalin-like substances in the blood were determined by the adsorption-calorimetric method [5] as modified by A. M. Utevskii [3]. As the main indicator of the changes in the proportions of adrenalin and adrenalin-like substances we took the coefficient of specificity.

Where the coefficient of specificity is equal to unity or less only adrenalin-like substances are present. Where the coefficient of specificity is equal to 2 or more only adrenalin is present.

Acetylcholine was determined by a biological method using the eserinized dorsal muscle of the leech. Determination of the potassium and sodium ions was carried out with a model 3 Zeiss flame photometer.

EXPERIMENTAL RESULTS

The results of the influence of centripetal acceleration acting in the caudal (head – pelvis) direction are shown in Table 1.

As it can be seen, with increase in the magnitude of the acceleration, starting from 4 g/ 30 sec, there is a significant rise in the content of adrenalin-like substances with complete disappearance of free adrenalin when the magnitude of the acceleration was 7-10 g/ 1 min. If, for instance, in the normal animal the content of adrenalin-like substances is 10.31 γ% and the coefficient of specificity 1.17, then after the action of acceleration of 10g/ 1 min the content of adrenalin-like substances was 12.5 γ% and the coefficient of specificity 0.85.

The acetylcholine content was slightly raised at an acceleration of 4g/ 15-30 sec; with an increase in the acceleration to 10 g the acetylcholine content fell. Thus, in the normal animal the acetylcholine content is 1.4 γ% and after the action of acceleration of 10 g/ 1 min it was 0.61 γ%.

* The accepted definition in aviation medicine of the direction of action of centripetal acceleration is in fact the direction of the opposing forces.

At the same time changes were observed in the content of potassium and sodium in the blood. After the action of acceleration of 10 g/1 min the sodium content fell slightly (from a normal value of 322.1 mg% to 310.6 mg%) while the potassium content rose (from a normal value of 25.6 mg% to 27.6 mg%). After the conclusion of the action of acceleration the acetylcholine and adrenalin-like substances in the blood were approximately at their initial level within 15-30 minutes.

TABLE 1

Results of the Action of Centripetal Acceleration in a Caudal Direction

Experimental conditions and direction of action of acceleration	Adrenalin-like substances		Acetylcholine (in γ%)
	in γ%	coefficient of specificity	
Control	10.31	1.17	1.40
Head – pelvis 4 g/15 sec*	8.30	1.20	1.60
" " 4 g/30 sec	9.80	1.00	1.51
" " 7 g/1 min	9.00	0.73	1.16
" " 10 g/1 min	12.50	0.85	0.61

*The fraction indicates that the acceleration acted for the time indicated in the denominator.

In a second series of experiments the effect of acceleration in a cranial (pelvis – head) direction was studied (Table 2).

TABLE 2

Results of the Action of Centripetal Acceleration in a Cranial Direction

Experimental conditions and direction of action of acceleration	Adrenalin-like substances		Acetylcholine (in γ%)
	in γ%	coefficient of specificity	
Control	10.31	1.17	1.40
Pelvis – head 4 g/15 sec	12.60	0.96	1.60
" " 4 g/30 sec	12.50	0.91	2.00
" " 7 g/1 min	12.90	0.84	1.30
" " 10 g/1 min	13.56	0.78	0.53

As can be seen in Table 2, the changes in the content of adrenalin-like substances and acetylcholine are somewhat more pronounced but are in the same direction as when the acceleration was applied in the caudal direction.

It must be pointed out that after the action of acceleration of a magnitude of 10 g/1 min in this direction the animals sometimes were in a comatose condition.

When acceleration was applied in a transverse direction (spine – sternum) in a magnitude of 10 g/1 min the acetylcholine content of the blood of the animals was practically unchanged and was at the upper limit of normal (1.52 γ%).

The changes in the content of acetylcholine and adrenalin-like substances and of electrolytes are due not only to the magnitude of the acceleration but also to the duration of its action. Thus, a study of the effect of acceleration acting in the caudal direction and of magnitude 4 g/5 min showed that in this case the acetylcholine content rose to 1.7 γ%, the sodium fell to 318.4 mg% and the potassium rose to 28.5 mg%.

In the final series of experiments which took into account the possibility of the cumulative effect of acceleration [2], we tried to ascertain the character of the changes in the content of the test drugs in the blood during repeated exposures to acceleration, deliberately making the experimental conditions severe.

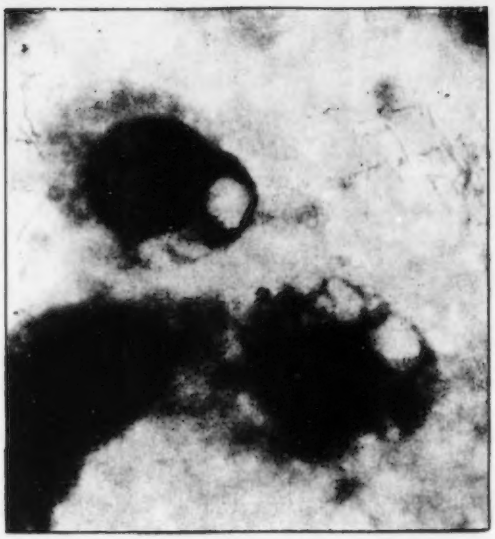

Fig. 1. Vacuolar degeneration of nerve cells in the brain of the rat after 120 acceleration procedures. Nissl's stain. Immersion.

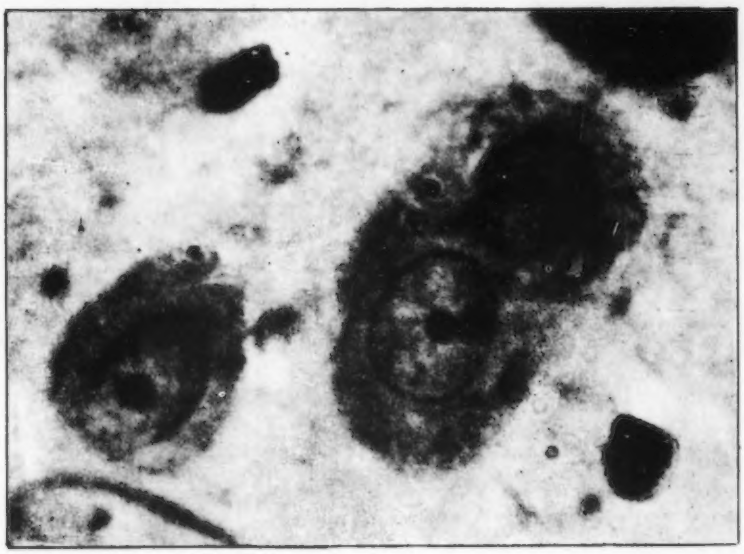

Fig. 2. Amitosis in a nerve cell in the brain of a rat after 120 acceleration procedures. Nissl's stain. Immersion.

The animals were subjected daily to four acceleration procedures in the head – pelvis direction and with a magnitude of 10 g/min. The intervals between the procedures was 30 minutes (Table 3).

TABLE 3

Results of the Repeated Action of Centripetal Acceleration in a Caudal Direction

Experimental conditions and direction of action of acceleration	Adrenalin-like substances		Acetyl-choline (in γ%)	Sodium (in mg%)	Potassium (in mg%)
	in γ%	coefficient of specificity			
Control	10.31	1.17	1.40	322.10	25.60
Head – pelvis 10 g/1 min	12.50	0.85	0.61	310.60	27.60
The same, after 32 procedures . . .	5.60	1.10	1.70	325.4	25.7
The same, after 120 procedures . .	4.80	2.60	1.38	309.00	26.50

As can be seen from Table 3, increase in the number of procedures to 120 leads to a considerable fall in the content of adrenalin-like substances (to 4.8γ %) and to a sharp rise in the coefficient of specificity (to 2.6). A fall in the sodium content (to 309 mg%) and a slight rise in the potassium content (to 26.5 mg%) are also observed. The acetylcholine content remains within normal limits.

In the experiments where 120 procedures were applied, morphological changes were found in different tissues of the body. In particular, cells with vacuolar degeneration and a considerable number of amitoses were found in the brain. Thickening of the vascular walls was also found in various organs (Figs. 1, 2 and 3).

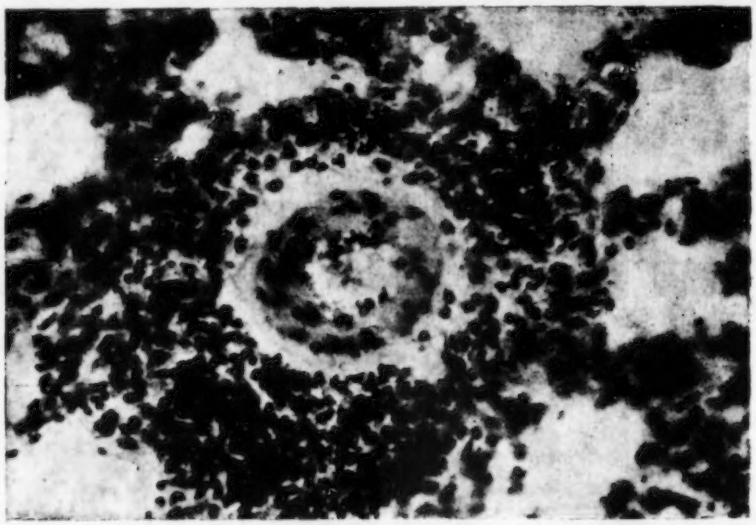

Fig. 3. Blood vessel with thickened walls in the lung of a rat after 120 acceleration procedures. Stained by hematoxylin-eosin. Magnification: ocular 10 ×, objective 40 ×.

The results of the experiments shown in this paper permit the role of the sympathetico-adrenal system in the mechanism of regulation of function during the action of acceleration on the body to be judged. Considerable importance here is attached evidently to noradrenalin which is one of the main pressor substances [4].

Changes in the acetylcholine content reflect not only changes in the excitability of the parasympathetic nervous system but also changes in the tone of the skeletal musculature. These findings are in agreement with observations made earlier by P. K. Isakov [1] and others who studied the bioelectric changes in skeletal muscle during the action of acceleration.

The occurrence of the most pronounced rise in the acetylcholine content with centripetal acceleration acting for a considerable length of time (5 minutes) and with a magnitude of 4 g suggests that under these conditions the compensatory tone of the muscle has reached its full development.

In our experimental conditions the changes in the contents of acetylcholine and adrenalin-like substances after single procedures are adaptive in character. After repeated procedures (32) a fall is observed in the content of adrenalin-like substances with a slight increase in the content of acetylcholine. After 120 procedures even more significant changes were found, demonstrating that the sympathetico-adrenal system is in a state of severe strain or even overstrain.

The morphological changes also indicate the presence of profound disturbances in the general condition of the animal arising after repeated acceleration procedures.

SUMMARY

The changes in blood content of adrenalin, acetylcholine, adrenalin-like substances, potassium and sodium were studied in animals after the action of centripetal acceleration. A definite dependance of changes in the content of these substances was noted on the value, direction and the time of action of accelerations. The morphological changes were also noted in various tissues of the body following numerous actions, of centripetal accelerations.

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BIOCHEMISTRY AND BIOPHYSICS

DISTRIBUTION OF TRACE ELEMENTS IN THE LUMBAR ENLARGEMENT OF THE SPINAL CORD FROM THE RESULTS OF SPECTRAL ANALYSIS

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The experimental data reported in the literature on the chemistry of the nervous system are mainly concerned with the chemical topography of the cerebral cortex [1, 2, 3]. Valuable results have been obtained by the method of spectral analysis [4, 5]. So far as the study of the chemical topography of other divisions of the central nervous system, for example the spinal cord is concerned, there are only isolated reports on highly specialized problems [1, 2].

We endeavored to study the distribution of the trace elements in the various regions of the lumbar enlargement of the spinal cord morphologically and functionally by the method of spectral analysis: in the grey and white matter, separately, in the grey matter of the posterior and anterior horns, and in the posterior and anterior roots.

EXPERIMENTAL METHOD

The experiments were performed on the spinal cords of cows obtained from the Dnepropetrovsk Meat combine from 7-12 minutes after the animals had been killed by severing the carotid arteries. The spinal cords were delivered to the laboratory of the Department in vacuum flasks on ice. For removal of weighed samples the spinal cord was placed ona watch glass, cooled on ice, and the dura and arachnoid taken away; afterwards the posterior and anterior roots were divided separately. Before removal of the samples from the cord, it was placed with its anterior surface uppermost. From the base of the anterior median fissure lateral incisions are made on both sides, 2-3 mm in depth, in order to detach the grey matter of the posterior horn and the tissue of the anterior horn, and the margins of the incision are carefully drawn aside. From above along the edges, the tissue of the grey matter of the anterior horns is laid bare, and in the center along both sides of the central canal – the tissue of the gray matter of the posterior horns, and underneath it, after removal of the grey matter, the tissue of the white matter of the spinal cord.

Samples of tissue weighing 1 g were dried in platinum crucibles at a temperature of 105°C to constant weight, then in the same crucibles they were incinerated in a muffle furnace at a temperature of 400-420°C for 8 hours.

The test samples were ignited in needle electrodes before the aperture of the spectrograph, the electrodes being cleaned by A. K. Rusanov's method [6]. The film was taken on a ISP-22 spectrograph. The source of excitation was a Svetnitskii activated arc; the generator a PS-39. The spectrum was deciphered on a spectro-projector PS-18 by means of S. K. Kalinin's atlas of spectral lines. Quantitative analysis of the trace elements in the samples was carried out by means of standards prepared by A. O. Voinar's method. The degree of blackening of the analysed lines was measured on a MF-2 microphotometer.

EXPERIMENTAL RESULTS

The results of qualitative analysis showed that the ash of the lumbar enlargementof the spinal cord and of

Concentration of Trace Elements in the Lumbar Enlargement of the Spinal Cord Calculated as Ash (in percent)

Area of the lumbar enlargement of the spinal cord tested	Lead	Chromium	Nickel	Aluminum	Manganese	Silicon	Titanium	Copper	Silver
The whole area	0.0003	0.001	0.001	0.0031	0.0005	0.001	0.002	0.0055	Traces
White matter	0.0019	0.001	0.001	0.0075	0.001	0.004	0.002	0.022	»
Gray »	0.0004	0.001	0.0015	0.0066	0.0015	0.002	0.003	0.003	»
« » of the anterior horn	0.0002	0.0005	None	0.0072	0.0005	0.003	0.002	0.002	»
« » of the posterior horn	0.0006	0.0014	0.001	0.0057	0.0025	0.001	0.002	0.004	0.001
Anterior root	0.0009	0.001	0.001	0.0038	0.0005	0.005	0.003	0.003	Traces
Posterior root	0.001	0.001	None	0.0006	0.0003	0.006	0.003	0.001	»

its emerging roots contains the following elements: calcium, potassium, magnesium, sodium, phosphorus, iron, chromium, aluminum, nickel, silver, titanium, copper, silicon, lead, manganese, zinc, cobalt and tin. The first six elements are found in larger quantities than the rest; they may be regarded as the macroelements of the spinal cord. Their quantitative analysis was not undertaken.

In view of the low threshold of sensitivity and the high volatility of zinc during ignition of the ash, its estimation by the method of emission spectral analysis was difficult. To estimate zinc a chemical method must be used. In estimation of cobalt it was found that its line was covered by lines from iron; this made the quantitative analysis of cobalt difficult.

The following trace elements were selected for quantitative analysis in the lumbar enlargement of the spinal cord: lead, chromium, nickel, aluminum, manganese, silicon, titanium, copper and silver. The results of the quantitative estimation of the trace elements in various areas of the lumbar enlargement of the spinal cord and the roots are shown in the table.

By examination of the numerical results relating to the individual trace elements we obtain the following.

Lead is uniformly distributed throughout all the tested areas of the lumbar enlargement of the spinal cord in an amount of 10^{-4}% calculated as ash, with the exception of the white matter and the posterior roots, where its concentration reaches 10^{-3}% calculated as ash.

Chromium is uniformly distributed throughout all the tested areas in a concentration of 10^{-3}% calculated as ash, with the exception of the anterior horn of grey matter, where it is slightly less.

Nickel is unevenly distributed. It is not found in the grey matter of the anterior horn nor in the posterior roots. In the remainder of the tested areas of the spinal cord the nickel content is 10^{-3}% calculated as ash.

Aluminum occupies the second place after copper in its content in the tested areas of the spinal cord. It is unevenly distributed. Its highest concentration (10^{-3}% calculated as ash) is in the white matter and in the grey matter of the anterior horns; it is slightly less in the grey matter (as a whole) and in the grey matter of the posterior horn. Its concentration is less by, roughly, one half in the tissues of the anterior roots and when calculated for the whole area of the spinal cord. Its concentration calculated as ash in the posterior roots is around 10^{-4}%.

Manganese is present in all the tested areas in a concentration of 10^{-3}% calculated as ash. In the grey matter of the posterior horn it is present in roughly 5 times the concentration in the grey matter of the anterior horn.

Silicon is concentrated mainly in the posterior and anterior roots and in the white matter in an amount of 10^{-3}% calculated as ash. This is evidently connected with the relatively high content of supporting tissue in these regions, characterized by a content of silicon.

Titanium is distributed uniformly throughout all the tested areas of the spinal cord in a concentration of 10^{-3}% calculated as ash.

The spinal cord is richest of all in copper. In the white matter its concentration is 10^{-2}% calculated as ash, and in other areas 10^{-3}% calculated as ash. The distribution of copper is as follows: relatively more in the grey matter of the posterior horn, slightly less in the anterior root, still less in the grey matter of the anterior horn and least of all in the posterior root.

Among the tested trace elements, silver was found to have the smallest concentration. In all the tested areas of the spinal cord only slight traces were found although in the grey matter of the posterior horn the silver content was 10^{-3}% calculated as ash.

According to their content of trace elements (in percent, calculated as ash) the tested areas of the lumbar enlargement of the spinal cord and the roots arising therefrom may be arranged in the following descending order:

White matter	0.0404
Grey matter (as a whole, without subdivision)	0.0190
Grey matter of the posterior horn	0.0183
Anterior root tissue	0.0182
Grey matter of the anterior horn	0.0154
Tissue of the whole area of the lumbar enlargement	0.0144
Posterior root tissue	0.0120

According to their quantitatively estimated concentrations (in percent as ash) in the lumbar enlargement of the spinal cord and in the roots, the trace elements tested may be arranged in the following order:

Copper	0.0060	Manganese	0.0010
Aluminum	0.0052	Nickel	0.0008
Silicon	0.0030	Lead	0.0007
Titanium	0.0020	Silver	0.0001
Chromium	0.0010		

It has thus been established by the method of emission spectral analysis that there is an unequal distribution and content of the trace elements in the lumbar enlargement of the spinal cord and in the roots arising from it.

SUMMARY

The quantitative content of microelements was investigated in various areas of the lumbar part of the spinal cord in cows. The method of spectral emissional analysis was applied. It was established that microelements (lead, chromium, nickel, aluminum, manganese, silicon, titanium, copper and silver) are distributed unevenly in the white matter, grey matter of the anterior horn, posterior horn, anterior and posterior roots of the spinal cord. The data which were obtained support the conception of Academician A. V. Palladin that brain areas which differ in their morphological structure and physiological function vary in their chemical content.

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THE AMMONIA AND GLUTAMINE CONTENT OF BRAIN TISSUE DURING ANEMIA RESULTING FROM LIGATION OF THE CAROTID ARTERIES*

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The present paper is devoted to the study of the effect of oxygen lack on the nitrogen metabolism of the brain. As an indicator of the state of the latter we took the content of preformed ammonia and the content of free glutamine in the tissue of the brain. According to a number of workers [10, 11, 12], anoxia is accompanied by stimulation of ammonia production in the brain. E. A. Vladimirova [2] found that with a profound degree of oxygen lack there is a fall in the ammonia production by the brain tissue.

EXPERIMENTAL METHOD

As experimental animals we took white rats weighing from 150 to 300 g. Incomplete anemia of the brain was obtained by application of ligatures to the common carotid arteries and subsequent division of these vessels. The operation was performed without general anesthesia. Cerebral anemia in rats is accompanied by two types of reaction [5, 7].

In one group of animals (convulsive group) periodic attacks of general motor excitation and convulsions developed, which were replaced by manifestations of severe inhibition. The other group of animals, after a brief period of gneral excitation, fell into a state of profound inhibition of the central nervous system (nonconvulsive group).

The brains of the convulsive group of rats were subjected to biochemical analysis 4-6 hours after operation, and those of the nonconvulsive group after 24 hours. These periods were selected in accordance with the observations made by K. G. Gromova et al. [5] in respect of changes in the metabolism of phosphorus compounds in the brain tissues of both groups of rats. The animals were frozen in toto in liquid oxygen during light ether anesthesia (5 minutes). Such brief anesthesia, as our control experiments showed, had no effect on the ammonia content of the brain. The tissue of the cerebral hemispheres, macerated in liquid oxygen, was treated with 5% trichloracetic acid to precipitate the proteins. The ammonia and glutamine content of the centrifugate were determined.

For determining the ammonia, we used the apparatus devised by S. D. Balakhovskii and Bruns [1] for estimation of the blood ammonia. This method was modified by us for application to brain tissue.

The ammonia was expelled from the centrifugate of brain after treatment with trichloroacetic acid by means of a borate buffer of pH 9.2-9.3 [2]. The ammonia content was estimated photometrically in the distillate with Nessler's reagent, using a graduated photometer or a photoelectric colorimeter M-1 with a blue filter. This method enables the ammonia content to be determined with an accuracy of a tenth part of microgram. The mean square error of the method is ± 4%.

* The substance of a dissertation submitted towards the degree of Candidate of Biological Sciences.

The brain centrifugate after trichloracetic acid treatment was hydrolyzed for 10 minutes with 2 N H_2SO_4 in a sealed tube on a water bath at 100°C [3] and the amide nitrogen of the glutamine was then estimated as ammonia.

EXPERIMENTAL RESULTS

Analysis of the brain tissue of normal healthy animals (10 white rats) showed that its preformed ammonia content averaged 0.43 mg% N—NH_3 with variation between 0.33-0.57 mg%, and the glutamine content averaged 7.08 mg% (6.21-9.04 mg%) N—NH_2.

Figures for the preformed ammonia content of the brain of white rats found in the literature vary considerably. Richter and Dawson [11] accept as normal 0.23 mg% N—NH_3 and 7.57 mg% N—NH_2 of glutamine, E. A. Vladimirova [2c] — 0.36 and 7.03 mg% and E. I. Gurina [1955] — 0.54 and 6.52 mg% respectively.

The control group of animals (7 white rats) was subjected to the same operation as the experimental animals up to the application of the ligatures to the vessels, but the ligatures were not tied nor the vessels divided. Until the time of removal of the brain for biochemical analysis (4-6 hours after operation for the convulsive group and 24 hours after for the nonconvulsive group), the behavior of the control rats was normal in every respect.

TABLE 1

Ammonia and Glutamine Content of the Brain of Control and Nonconvulsive Groups of Rats 24 Hours After Operation (in mg% nitrogen per dry weight of tissue)

Control animals			Animals with induced anemia		
Experiment No.	N—NH_3	N—NH_2	Experiment No.	N—NH_3	N—NH_2
38	0.34	8.59	24	0.50	6.70
			30	0.46	8.30
			41	0.49	9.01
			42	0.57	7.27
40	0.40	8.38	44	0.57	7.31
			72	0.85	10.39
			75	0.80	10.02
61	0.40	9.87	96	0.47	8.36
			97	0.48	6.07
			99	0.45	8.19
Average	0.38	8.94	Average	0.57	8.16

The investigation (Table 1 and 2) showed that operative trauma causes slight changes in the content of the nitrogen-containing compounds under study in the brain tissue. In the 4-6 hour control the ammonia content was on the average 0.56 mg% of nitrogen (normal 0.43 mg%), but 24 hours after operation it had returned to normal (0.38 mg%). A slight rise in the glutamine content was observed in the 4-6 hour period (7.97 mg% N—NH_2) with a further rise at 24 hours to 8.94 mg%. In this connection we compared the results of the experiments on the rats with induced anemia with the values obtained in control experiments.

The nonconvulsive group of rats with induced anemia fell after operation into a state of severe inhibition and 24 hours later they began to show trophic disturbances in the form of blepharoconjunctivitis and falling out of the fur. In the brain of these animals 24 hours after operation an increase in the ammonia content by 50% over the controls was found. The changes in the glutamine content were inconstant in character and on the average they showed a slight tendency to fall (see Table 1).

The other group of animals reacted to the production of cerebral anemia by intense excitation and by the periodic occurrence of convulsions, in the intervals between which the rats were in a state of complete prostration. Their body temperature fell sharply.

In these experiments (Table 2) the mean content of preformed ammonia was increased more than 3 times over the control values. The ammonia content was particularly high (more than 4 times higher than the control) in animals with very severe convulsive attacks (experiments Nos. 102, 104, 105). In experiments Nos. 104 and 105 the rats were frozen in a convulsive state and in experiment No. 102 in a state of intense excitation, wich usually preceded a convulsion. The glutamine content of these animals was found to be less than the control value. A particularly sharp fall (by 30-35%) was observed in experiments Nos. 104 and 105.

TABLE 2

Ammonia and Glutamine Content of the Brain of Control and Convulsive Groups of Rats 4-6 Hours after Operation (in mg% of nitrogen per dry weight of tissue)

Control animals			Animals with induced anemia			State of the animal at the moment of removal of the brain for biochemical analysis
Experiment No.	N—NH_3	N—NH_2	Experiment No.	N—NH_3	N—NH_2	
80	0.64	7.42	31	0.77	8.35	Killed in the interval between convulsions in a state of inhibition
86	0.63	9.60	33	1.41	8.76	
			79	1.71	9.49	The same
91	0.57	7.97	102	2.08	7.09	Killed at a period of general excitation
103	0.43	6.90	104	2.08	5.69	Killed at a period of very strong convulsions
			105	2.55	5.19	
Average	0.56	7.97	Average	1.77*	—*	

*In view of the different trends in the changes in the glutamine content of the brain of the convulsive group of animals, mean values of this quantity are not deduced.

In the rats killed during a convulsion-free period, in a state of inhibition (experiments Nos. 31, 33, 79) the glutamine content was 5-20% higher than the control value.

Investigation of the brain tissue of the nonconvulsive group of rats from 4-6 hours after operation showed that changes in the ammonia and glutamine contents which could be attributed to anoxia could not be found at this period; they appeared only after 24 hours.

The results obtained show that during an inadequate supply of oxygen to the brain an increase takes place in its content of preformed ammonia. This rise was particularly noticeable in cases where the oxygen lack is aggravated by general excitation of the central nervous system and by convulsions. As V. I. Rozengart [9] showed, convulsions due to pharmacological agents lead to merely a very transient rise in the ammonia content of the brain, in spite of the continuing convuslive attacks, and do not cause changes in the glutamine content. Consequently, the marked and persistent increase in ammonia and the fall in the glutamine observed in the convulsive group are the results of anoxia.

The increase in the ammonia content of the brain under the experimental conditions studied may be attributed to stimulation of deamination processes. In anoxia there is a disturbance of oxidative phosphorylation, accompanied by breakdown of ATP with the formation of adenosinediphosphate and adenosinemonophosphate [4, 8]. The latter may act as a source of the ammonia in the brain during its oxygen lack.

Free glutamine, according to E. A. Vladimirova's findings [2b], also takes part in the formation of ammonia. It is possible that in our experiments also, part of the ammonia formed in the brain arises in consequence of glutamine breakdown, for the content of glutamine is in fact greatly reduced during aggravation of anoxia.

Alongside the other sources of ammonia and probably the most potent must be mentioned the amide groups of the proteins of nerve tissue [3]. It may be that in oxygen lack, expecially if accompanied by excitation of the central nervous system, the deamination of proteins in the brain is intensified, leading to the accumulation of considerable quantities of ammonia.

On the other hand, the increase in the ammonia content of the brain tissue during anoxia may also be explained by slowing of its removal through glutamine synthesis on account of the deficiency of ATP. In support of this hypothesis there is the reduction in the content of free glutamine which was found under these conditions, especially pronounced in the brains of the convulsive group of rats.

During oxygen lack, glutamine metabolism in the brain, as shown above, is disturbed. When anoxia develops slowly (in the nonconvulsive group of rats) the changes in glutamine metabolism are indistinctly expressed – there is a tendency for its content to fall. If this pathological process runs an acute course (in the convulsive group) this fall in the glutamine content is very marked. We consider that the fall in the glutamine content of the brain during anoxia may possibly be accounted for by interference with its synthesis from glutamic acid and ammonia in connection with the lack or even the total absence (in the convulsive group) of ATP, found in rats after ligation of the carotid arteries [4]. The small rise in the glutamine content found in isolated experiments may probably be explained in these animals by the particularly profound inhibition in the central nervous system, causing a certain predominance of processes of synthesis.

TABLE 3

Ammonia and Glutamine Content of the Brain of the Nonconvulsive Group of Rats (in mg% of nitrogen per dry weight of tissue) 4-6 Hours After Operation

Control animals (average of five experiments)		Experiment No.	Animals with induced anemia	
$N—NH_3$	$N—NH_2$		$N—NH_3$	$N—NH_2$
0.59	7.51	93	0.39	7.61
		94	0.44	7.75
		98	0.77	7.16
		Average	0.53	7.50

Changes which we discovered in the indices of nitrogen metabolism in the brain that we investigated during cerebral anemia may, we consider, be explained not only by oxygen lack but also by interference with the supply of nutrient substances and energy-producing materials to the brain and with the removal of metabolic products, which result from the disturbance of the cerebral circulation. Summing up, we may conclude that during deficiency of oxygen in the brain, caused by incomplete anemia, the quantity of preformed ammonia in the brain is increased in consequence of breakdown of ammonia-forming compounds during anoxia and of delay in the removal of the ammonia formed.

In glutamine metabolism under these conditions breakdown is slightly in excess of the processes of synthesis.

Convulsions in association with disturbance of the cerebral circulation aggravate these changes in the nitrogen metabolism of the brain: the ammonia content is increased particularly sharply and the glutamine content shows a marked fall.

SUMMARY

The author studied the effect of oxygen insufficiency occuring in the brain in "exclusion" of both common carotid arteries on the content of ammonia and glutamine in it. Ammonia was determined by the Balakhovskii-Bruns method modified by us for brain tissue, while amide nitrogen of glutamine was determined by ammonia content after 10-minute hydrolysis with 2 N H_2SO_4 at 100°C.

The brain tissue of white rats reacted to anemia by widespread stable inhibition. Increase in the quantity of ammonia by 50%, as compared to control, was revealed in the central nervous system. The glutamine content tends to decrease. These changes were more pronounced in the brain tissue of rats in which brain anemia is asso-

ciated with attacks of general excitation and cramps. The quantity of ammonia in this case increased 3 times, while the amount of glutamine is decreased by 30-35% in comparison with control values.

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PHARMACOLOGY

CHANGES IN ARTERIOVENOUS ANASTOMOSES CAUSED BY THE ACTION OF CERTAIN DRUGS

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The aim of the present investigation was to study the function of arteriovenous anastomoses and in particular to discover the mechanism of regulation of changes in their lumina.

The state of arteriovenous anastomoses was determined by means of our modification of the method of microscopy of vessels in vivo, known in the literature as the method of "Clark's windows" (1931 [4]).

The essence of the method is that following careful dissection and excision of the cartilage from part of the ear of the rabbit a translucent layer of tissue is created, containing undamaged vessels with their innervation intact.

This area is enclosed in a transparent chamber made of organic glass,* which permits observations to be made on the vessels by transmitted light for periods of weeks or months under the microscope.

We measured the diameter of the vessels by means of an ocular micrometer and the observations were recorded by means of a "Kolibri" microcamera [1].

Observations on the vessels in vivo made it possible to judge changes in their diameter, the character of contractions of the vascular wall and also the rate and direction of the blood flow.

Observations were made on 26 rabbits undergoing this form of operation. The duration of the observations on the vessels in the chambers varied from 3 weeks to 1½ months. Each experiment lasted for one to 3-4 hours.

In order to study the mechanisms of regulation of the activity of the arteriovenous anastomoses, on the recommendation of S. V. Anichkov we used adrenalin, sympatholytin and hexonium, which interrupt the vegetative reflex arc at different points.

Experiments Using Adrenalin

In order to determine the character of the effect of stimulation of the sympathetic nerves on arteriovenous anastomoses adrenalin was used.

The effect of adrenalin on the arteriovenous anastomoses of the rabbit's ear has been studied by many workers [5, 6, 7]. According to all these writers adrenalin produced closure of the lumina of the arteriovenous anastomoses.

We injected rabbits subcutaneously with adrenalin in a dose of 0.3 to 1.1 mg per 1 kg body weight (22 experiments), usually using a dose of 1 mg.

As a rule the arteriovenous anastomoses reacted to the injection of adrenalin immediately. In the 2nd minute after injection their lumina began to diminish. The length of the period of closure of the anastomoses increased and their lumina rapidly disappeared completely. The arteriovenous anastomoses remained in a spastic state for a long time – for 2 hours and more after injection. The arterioles began to react to the injection of adrenalin somewhat later, and their diameter was reduced by an average of 70%.

* Plastic material used in the USSR.

The reaction of the venules to the injection of adrenalin was weaker than that of the arterioles – on the average the diameter of the venules was diminished by 45%.

Fig. 1. Unequal contraction of the walls of the arteries of the rabbit's ear under the influence of adrenalin (0.1 mg/kg body weight). Microphotograph. Magnification: objective 8 ×, ocular 4 ×.

At the outset a slight, transient increase in the contractile activity of the arterioles could be observed, after which they became greatly constricted. In some cases the appearance of well-defined thickenings against a background of spastic contractions of the arterioles could be observed, and sometimes in the arteries also (Fig. 1). It must be emphasized that observations of this sort apply only to the arterial vessels, the lumen of the veins being constricted uniformly throughout their entire length after the injection of adrenalin. We were unable to find in the literature any description of observations of this sort.

The difference in the tone of the walls of the arterial vessels during the action of adrenalin is difficult to explain. We can only make the suggestion that adrenoreactive biochemical structures are unequally distributed throughout the arterial vessels.

Experiments Using Sympatholytin

Sympatholytin possesses a marked sympatholytic and adrenolytic action. The effects of sympatholytin are explained by its power to block the adrenoreactive biochemical structures of the tissues, with which the mediator of the sympathetic fibers and the adrenalin of the blood normally react [3].

We injected a 3% alcoholic solution of sympatholytin in 5 ml of physiological saline in a dose of 15-20 mg/kg body weight of the animals intravenously into the marginal vein of the rabbit's ear (11 experiments).

Within a few seconds of the injection of the sympatholytin solution the arteriovenous anastomoses which previously were closed were opened widely and simultaneously those which were already open were dilated still further.

As a rule the anastomoses remained maximally dilated (their diameter increased by 400-600%) for a period of $1\frac{1}{2}$ - 3 hours. The arteries and arterioles were considerably dilated – their diameter was increased 2 - $2\frac{1}{2}$ times, whereas the diameter of the venules increased by only $1\frac{1}{2}$ times. A clear acceleration of the blood flow through the vessels of the ear was also observed.

Simultaneously with the rapid and considerable dilatation of the arteries and arterioles very frequent pulsations appeared in their walls – over 200 pulsations in one minute, coinciding with the number of contractions of the heart. The amplitude of these pulsations usually did not exceed 10-15 μ.

Experiments Using Hexonium

In order to exclude pharmacologically the vasomotor neurone in the region of the synapses between the pre- and postganglionic sympathetic fibers we used hexonium which has the property of blockading vegetative ganglia, mainly sympathetic [2]. Hexonium was injected subcutaneously as a 4% aqueous solution (24 experiments).

It was discovered that hexonium in doses of 3-25 mg/kg body weight produces only slight acceleration of the blood flow and only in isolated experiments was slight dilatation of the anastomoses, arterioles and venules observed. Doses of hexonium from 30 mg/kg body weight and over resulted in obvious dilatation of the vessels. Administration of hexonium in a dose of 80 mg/kg body weight caused death of the animals.

The arteriovenous anastomoses and vessels of the ear began to dilate under the influence of hexonium after 2-8 minutes, reaching maximum dilation 12-27 minutes after the moment of injection of the drug. Gradually more and more of the arteriovenous anastomoses opened up; the lumina of those anastomoses which could be seen even before injection of hexonium increased considerably (Fig. 2). At the same time the anastomoses opened up the diameter of the arteries and arterioles increased (on the average by 130%), as did also that of the venous vessels (on the average by 80%).

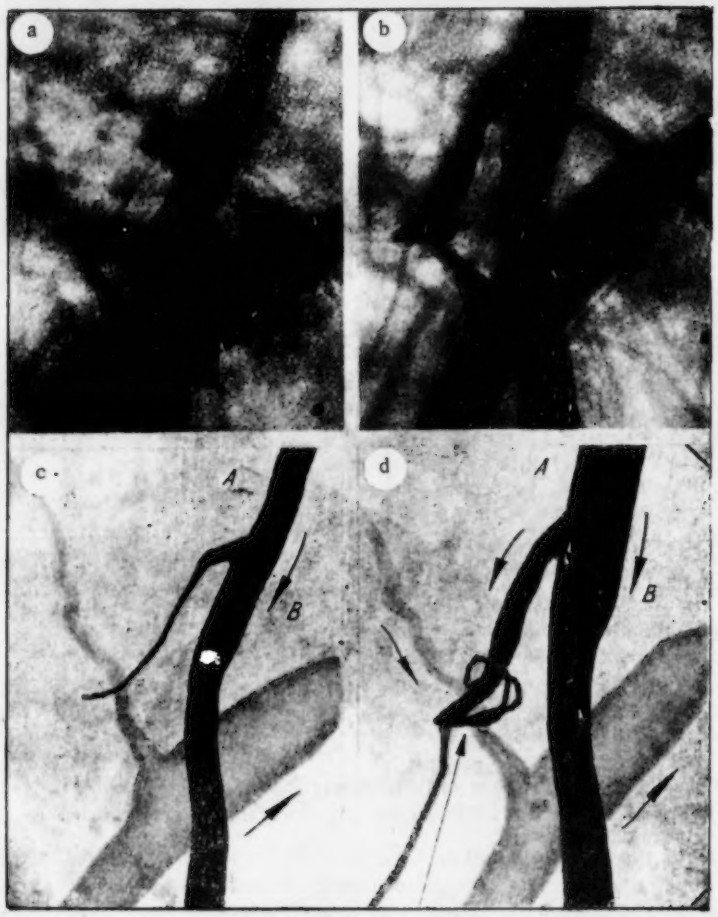

Fig. 2. The effect of hexonium on the arteriovenous anastomoses and vessels of the rabbit's ear.
a) Before injection of hexonium; b) 20 minutes after injection of hexonium (33 mg/kg); c and d) outlines of the microphotographs. The same magnification as in Fig. 1.

The duration of the vasodilatory action of hexonium in a dose of 33 mg/kg varied between $2\frac{1}{2}$ and 4 hours.

The experiments using hexonium clearly showed that the resulting fall in the tonus of the sympathetic nerves was accompanied by opening up and dilatation of the arteriovenous anastomoses, and increase in the diameter of the arterial and venous vessels.

SUMMARY

The author studied the function of arteriovenous anastomoses with the aid of a modified method of direct microscopy of the rabbit's ear vessels in vivo. Changes of the diameter of vessels was evaluated with the aid of

the ocular micrometer and were registered microphotographically. Adrenalin, sympatholytin and hexonium were used for investigation of the mechanisms of regulation of activity of arteriovenous anastomoses. The results of observations allowed us to conclude that the main factor regulating the function of arteriovenous anastomoses is the increase or decrease of the tone of the sympathetic innervation.

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EXPERIMENTAL BIOLOGY

A STUDY OF THE ANTIGENIC PROPERTIES OF TISSUES AND ORGANS OF ANIMALS DURING ONTOGENESIS

COMMUNICATION VI. THE STAGE-SPECIFIC ANTIGENS OF THE CHICK'S HEART

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In a previous paper [3] results were described which demonstrated that the tissues of the developing crystalline lens of the duck in early stages of embryogenesis (90-130 days of incubation) contain antigens which are characteristic of these early stages of development only, and subsequently disappear. We called these antigens "stage-specific." In the same article a number of reports were quoted from the literature which indicated the existence of certain antigenic substances only at definite stages of development.

We were able to find only one paper [5] in the literature in which the authors reported the presence of the muscle tissue of rabbit and sheep embryos of a contractile protein which was characteritic of the embryonic period of development and which was called "metamyosin" by these authors. It must be mentioned that somewhat earlier I. I. Ivanov and B. S. Kasavina [2] showed a qualitative change in the contractile proteins during ontogenesis.

In the present paper a description is given of antigens which are characteristic of definite stages of development of the chick's heart.

EXPERIMENTAL METHOD

We used the reaction of anaphylaxis with desensitization in guinea pigs [1]. In the first series of experiments we investigated the presence in the tissues of the developing heart of antigens which were characteristic of definite stages of development. The guinea pigs were sensitized subcutaneously with suspensions of heart tissue of chick embryos ("Russian White" breed) with different periods of incubation (4, 6, 8, 10, 12, 16 and 19 days) and of 3-day chicks. The heart tissue was thoroughly and repeatedly washed free from blood in physiological saline. The washing waters were examined for serum protein content by means of the ring-precipitation test. On the 21st day after the sensitizing injection all the animals were desensitized to species- and organ-specific antigens by intravenous injections of tissue extracts from the heart of an adult chicken (400 mg each).

Desensitization to organ-specific antigens of the heart arose very slowly in our experiments. For this reason, in a number of cases the animals had to be given 3-4 intravenous injections of tissue extract from the heart of an adult chicken, and only then did they become desensitized to the organ-specific antigens. Two hours after testing the completeness of desensitization to these antigens (intravenous injection of tissue extract of the heart of a chick in a dose of 500 mg) the animals were injected intravenously with the same antigens which were used to cause sensitization. The only difference was that extracts and not suspensions of the tissue were injected.

The extract was obtained in the following manner. The heart tissue was washed free from blood and then quickly ground in a homogenizer together with 4 volumes of cold physiological saline. The suspension obtained was centrifuged in the cold form for 20 minutes at a rate of 3000 rpm.

TABLE 1

The Reaction of Anaphylaxis in Guinea Pigs Sensitized by Suspensions of Heart Tissue of Chick Embryos and Desensitized to Species- and Organ-specific Antigens, in Response to Injection of a Tissue Extract of the Heart of Embryos of Various Periods of Incubation

Sensitization (subcutaneously)		Assaulting injection (intravenous) after complete desensitization to species- and organ-specific antigens		
antigen	dose in mg	antigen	dose in mg	reaction
1. Tissue suspension of the heart of chick embryos of the following periods of incubation (in days)		1. Tissue extract of the heart of chick embryos of the following periods of incubation (in days)		
4	16	4	400	+
4	16	4	400	+
4	16	4	400	+
6	16	6	400	—
6	16	6	400	—
6	16	6	400	—
8	16	8	400	—
8	16	8	400	—
8	16	8	400	—
10	16	10	400	±
10	16	10	400	+
10	16	10	400	±
12	16	12	400	+
12	16	12	400	+
12	16	12	400	+
12	16	12	400	++
12	16	12	400	+
12	16	12	400	+
16	16	16	400	++
16	16	16	400	+
16	16	16	400	++
19	16	19	400	+
19	16	19	400	+
19	16	19	400	+
2. Tissue suspension of the heart of a 3-day chick	16	2. Tissue extract of the heart of a 3-day chick	400	±
The same	16	The same	400	+
» »	16	» »	400	±
3. Technical control (sensitization not produced)	—	3. Tissue extract of the heart of chick embryos of 4-days of incubation	400	—
The same	—	The same	400	—
» »	—	4. Tissue extract of the heart of chick embryos of 12 days of incubation	400	—
		The same	400	—

Signs: + tremor, rubbing of the nose and ears, dishevelling of the fur, shortness of breath, slight fall of temperature; ++ the same signs, more severely shown, the guinea pig often sneezes; ± signs of anaphylactic shock are shown indistinctly; — no signs of shock.

EXPERIMENTAL RESULTS

A positive anaphylaxis reaction was observed in guinea pigs sensitized with suspensions of heart tissue of embryos of 4, 12, 16, and 19 days incubation after the injection of extracts from heart tissue of embryos (Table 1). Meanwhile, in guinea pigs sensitized with suspensions of heart tissue of embryos of 6 and 8 days incubation no signs of anaphylactic shock were observed. After injection of the assaulting dose of antigen (tissue extracts from the hearts of 4- and 12-day embryos) into nonsensitized animals (technical control) no positive anaphylactic reactions were obtained. In guinea pigs sensitized with suspensions of heart tissue of embryos of 10 days incubation and of 4-day chicks the results were mainly indefinite (±). The guinea pigs sensitized with tissue suspensions of the heart of embryos of 4, 12, 16 and 19 days of incubation gave a marked anaphylactic reaction which was assessed as + and ++.

The results obtained demonstrate that the tissues of the developing heart at a definite period of embryogenesis contain antigens which are specific for that stage of development alone — stage-specific antigens. These antigens are present in the heart tissue of embryos of 4 days of incubation, disappear in embryos of 6 and 8 days of incubation, reappear in embryos after 10 days of incubation and disappear once more after hatching.

During embryogenesis, therefore, 2 periods are observed in the course of which it is possible to find stage-specific antigens. The question naturally arises at once, are these the same stage-specific antigens or are the disappearing stage-specific antigens replaced in the course of development by new antigens. In order to answer this question we carried out a second series of experiments.

Four guinea pigs were sensitized by tissue suspensions of the heart of embryos of 4 days of incubation. On the 21st day after the sensitizing injection all the animals were desensitized to species-specific (by intraperitoneal injection of a dose of 500 mg of chick serum) and organ-specific antigens (by intravenous injection of tissue extract of chick's heart in a dose of 500 mg). Two hours after testing for complete desensitization to organ-specific antigens all the animals were injected intravenously with 500 mg each of tissue extract of the heart of embryos of 14 days of incubation. In no animal was a positive anaphylactic reaction observed (Table 2). Thus, the results of this series of experiments demonstrate that the stage-specific antigens present in the heart tissues of 4-day chick embryos are different from the stage-specific antigens found in the heart tissues of embryos of 12-19 days of incubation.

TABLE 2

The Reaction of Anaphylaxis in Guinea Pigs Sensitized by Suspensions of Heart Tissue of Chick Embryos of 4 Days of Incubation and Desensitized to Species- and Organ-specific Antigens, in Response to the Injection of a Tissue Extract of the Heart of Embryos of 14 Days of Incubation

Sensitization (subcutaneously)		Assaulting injection (intravenous) after complete desensitization to species- and organ-specific antigens		
antigen	dose in mg	antigen	dose in mg	reaction
Tissue suspension of the heart of embryos of 4 days of incubation	16	Tissue extract of the heart of embryos of 14 days of incubation	500	—
The same	16	The same	500	—
" "	16	" "	500	—
" "	16	" "	500	—

The same signs as in Table 1.

Arising from these findings it appears that the tissues of the developing chick's heart contain 2 different groups of stage-specific antigens which are present at different stages of development.*

In 1952, Schjeide [6] reported that in the serum of chick embryos of the 10th day of incubation antigens

* It is possible that in this particular case the tissues contain only one stage-specific antigen. However, lacking appropriate accurate data, we shall speak of groups, without begging the question of the number of component antigens.

were present which were not to be found in the serum of the adult bird. In view of the extremely high sensitivity of the anaphylactic reaction, we began to wonder whether the stage-specific antigens present in the heart tissues of 12-19-day embryos were not the antigens of the blood serum of embryos of these same periods of incubation. We thought that despite the thorough washing of the pieces of heart tissue to free them from blood serum, some small trace of serum proteins may nevertheless remain and thereby bring about sensitization of the animals to these antigens. In order to answer this question we made a comparative study of the antigenic properties of the heart tissue and the serum of embryos of 14 days of incubation. In the heart tissue of embryos of this period of development there is evidently the greatest number of Group II stage-specific antigens. In the first place it was necessary to find out whether stage-specific antigens are present in the serum of embryos of 14 days of incubation, and if so, in what amount.

With this object we carried out a third series of experiments. Four guinea pigs were sensitized subcutaneously with serum from embryos of 14 days of incubation. On the 21st day after the sensitizing injection all the guinea pigs were desensitized to the antigens of the serum of the adult chicken, after which they were injected intravenously with the serum of 14-day embryos. In addition the same antigen was injected into 3 nonsensitized guinea pigs (technical control).

The results of the experiments showed (Table 3) that 3 guinea pigs gave a positive anaphylactic reaction in response to the injection of the serum of 14-day chick embryos and in one guinea pig signs of anaphylactic shock were indistinct (±). Meanwhile no positive reaction was observed in the control animals. Thus, the results of the experiments showed that in the blood serum of embryos of 14 days of incubation stage-specific antigens are present in small quantity.*

In order to solve the problem whether Group II stage-specific antigens are present in the heart tissues or whether this group is a mixture of serum antigens, we carried out a fourth series of experiments. Four guinea pigs were sensitized with suspensions of thoroughly washed heart tissue from embryos of 14 days of incubation, free from blood serum. On the 21st day after the sensitizing injection the animals were desensitized to species and organ--specific antigens by the intraperitoneal injection into each guinea pig of 500 mg of serum and 400 mg of extract of heart tissue of an adult chicken. Two hours after testing the completeness of desensitization to organ-specific angigens (by intravenous injection of an extract of the heart tissue of the chicken in a dose of 500 mg) all the guinea pigs were injected intravenously with an extract of the heart tissue of 14-day embryos. In addition,this same antigen was injected into 3 nonsensitized guinea pigs (technical control).

The results of the experiment showed (Table 3) that in all the experimental guinea pigs a well-marked positive anaphylactic reaction was observed in response to the injection of an extract of heart tissue of 14-day embryos. In the control guinea pigs no signs of anaphylactic shock were observed. Thus, the results of the fourth series of experiments showed that the heart tissues of embryos of 14 days of incubation contain a considerable quantity of the stage-specific antigens.

As has already been pointed out above, in the third series of experiments as sensitizing and assaulting injections we used the serum of embryos of 14 days of incubation,but in the fourth series of experiments, suspensions and extracts of the heart tissues of embryos of the same period of development. Contamination by serum antigens of the suspensions and extracts used in the fourth series of experiments was evidently very slight. However, the degree of the anaphylactic reaction to stage-specific antigens in the guinea pigs of the fourth series of experiments was higher than in those of the third series. It therefore may be thought that the positive anaphylactic reaction in the animals of the fourth series of experiments was caused by the stage-specific antigens present in the heart tissues of 14-day embryos and not by mechanical contamination by the antigens of the serum of the same embryos.

A morphological study which we carried out showed that in the period when the Group I stage-specific antigens are found,the process of transformation of the tubular heart into a four-chambered organ is beginning. At this period of development a high tempo of growth is observed in the heart tissues, and in its histological structure the myocardium resembles in its main features the myocardium of embryos of earlier periods of incubation (there

* As a result of cross anaphylactic reactions with antigens of serum and heart tissue of 14-day embryos it was shown that the stage-specific antigens of the serum and also of the heart tissue of the embryos are evidently qualitatively the same. However, the qualitative similarity of the antigens could not serve as a convincing argument in support of any theory of their localization.

are many mitoses in the myocardium and it has a syncytial cell structure with a small number of transversely striated myofibrils). It may be thought that the presence of stage-specific antigens in the heart tissues of embryos of 4 days of incubation is connected with the particular morphological and physiological features of development of the heart in early stages of embryogenesis, when it is still tubular in structure. We may point out that the susceptibility of the developing heart of the chick embryo to external agents changes considerable after 4 days of incubation [8].

TABLE 3

The Reaction of Anaphylaxis in Guinea Pigs Sensitized with Serum and Suspensions of Heart Tissue of Chick Embryos of 14 Days of Incubation and Desensitized to Species- and Organ--specific Antigens, in Response to the Injection of Serum and of Extract of Heart Tissue of Embryos of the Same Period of Incubation

Sensitization (subcutaneously)		Assaulting injection (intravenous) after complete desensitization to species- and organ-specific antigens		
antigen	dose in mg	antigen	dose in mg	reaction
1. Serum of chick embryos of 14 days of incubation . . .	100	1. Serum of chick embryos of 14 days of incubation		
The same	100	The same	400	+
" "	100	" "	400	±
" "	100	" "	400	+
2. Technical control (sensitization not produced)		" "	400	+
		" "	400	−
3. Suspension of heart tissue of 14-day chick embryos. . .	16	" "	400	−
The same	16	" "	400	−
" "	16	2. Extract of heart tissue of chick embryos of 14 days of incubation.	500	+
" "	16	The same	500	++
" "	16	" "	500	++
4. Technical control (sensitization not produced)	−	" "	500	+
The Same	−	" "	500	−
		" "	500	−
		" "	500	−

The same signs as in Table 1.

It has not yet been possible to observe what these definite morphological peculiarities in the development of the heart may be, in the period when the Group II stage-specific antigens are found (12-19 days of incubation). However there are reports in the literature which demonstrate that at this period of development the heart tissue is undergoing essential biochemical changes. Thus, for instance, Moog [4], when studying the activity of apyrase (adenylpyrophosphatase) in the developing cardiac muscle of chick embryos beginning with 12 days of incubation, established that the apyrase activity rises sharply on the 16th day of incubation and then falls again at the moment of hatching. The apyrase activity in embryos of 16 days of incubation is 400% higher than that of 12-day embryos. These findings show that the intensity of metabolism in the heart muscle of embryos of 16 days of incubation is increased. Similar results showing increased enzyme activity in cardiac muscle during the second period of embryonic development were obtained by Sippel [7] with another enzyme – succino-dehydrogenase. It is possible that the presence of Group II stage-specific antigens in the developing heart is connected with the particular biochemical changes in the tissues at this period of development.

Thus, as a result of a study of the antigenic properties of the developing heart, 2 groups of stage-specific antigens have been demonstrated. The presence of stage-specific antigens in the heart tissues is connected with the particular morphological and physiological features of development of this organ at the various stages of ontogenesis.

SUMMARY

Anaphylactic reaction with densensitization was employed in guinea pigs to show that there are 2 different groups of stage-specific antigens in the chick's heart. The first group of antigens is present in the embryonic cardiac tissues during the first 4 days of incubation, while the second appears in the developing heart 10 days after incubation and disappears after hatching. Existence of stage specific antigens in the cardiac tissues is connected with definite morphologico-physiological peculiarities of development of this organ in different stages of ontogenesis.

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ONCOLOGY

METASTASIS OF SARCOMA M-1 IN RATS AND THE BIOLOGICAL PROPERTIES OF THE METASTASES

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We are not the first to study the character of growth of transplanted tumors in relation to the site of inoculation. This problem appeared earlier in work by Keysser [1911], Endler [1916] and other investigators who showed that tumors grow in different ways depending on the site of inoculation.

Recently Soviet workers in this direction have established a number of interesting features of tumor growth. A. A. Solov'ev, for example, studied the principles of spread of metastases of the Brown-Pearce tumor of rabbits and their relation to the site of development of the primary tumor. This worker injected a suspension of tumor cells into the testis, ovary, kidney, adrenals, liver, spleen, retrobulbar tissues and the anterior chamber of the eye. In this way he observed that, depending on the site of injection of the tumor, the pattern of metastasis formation was varied. O. I. Epifanova [1] in her research showed that the Ehrlich adenocarcinoma of mice grows best in the region of the forelimbs and worst in the hindlimbs. After subcutaneous inoculation this tumor as a rule does not form metastases but after intraperitoneal inoculation an ascitic variant of the malignant cells develops, in a certain percentage of cases with metastases in the liver, kidneys, spleen and other organs. The M-1 rat sarcoma, also inoculated subcutaneously, as a rule does not give metastases. However V. N. Popov [3] observed the development of metastases from this tumor in the internal organs after its inoculation in the muscle tissue of the hind limb or into the testis.

Similar results were obtained by Hlavayova and Kossey [8] with inoculation of the rat tumor BSH-1 into the testicle of experimental animals. As another example confirming the ability of tumors to grow in different ways depending on the site of inoculation may be mentioned the ascitic variants of malignant cells which are formed by intraperitoneal passage through experimental animals of subcutaneous or solid forms of tumors [7, 9, 11, 12, 13].

In view of these findings we decided to study the possibility of metastasis formation by the rat sarcoma M-1 after inoculating the tumor into rats subcutaneously and into the testicle, and also the biological properties of the metastatic tumor tissue after subcutaneous inoculation of experimental animals with it.

The rat sarcoma M-1 was first obtained by L. M. Shabad [5] by injecting rats with a solution of 3 : 4 benzpyrene in castor oil. According to the findings of this worker this tumor, from a histological point of view, is a spindle-cell and partly polymorphocellular sarcoma with low malignancy and a low percentage of successful inoculation (50%).

In our experiments the experimental animals used were sexually mature males aged from 6-10 months. In all the experiments we carried out the inoculation with tumor tissue with strict sterile precautions and with the same dosage: 0.5 ml of a 10% suspension of tumor particles in physiological saline.

EXPERIMENTAL RESULTS

Inoculation of the Sarcoma M-1 Subcutaneously

For subcutaneous inoculation 112 rats were used. Of these, 74 rats died from the developing tumors (66%),

in 15 rats the transplants at first showed some degree of growth and then regressed, while in 23 rats the transplantation was unsuccessful.

During pathological anatomical investigations of the experimental animals dying from tumors we never once found in them any metastases in the internal organs or peritoneal cavity. The tumor nodules grew in the form of continuous conglomerating masses, sometimes reaching large dimensions and a weight of 140 g or more. Usually in the depth of these tumor nodules could be found a cavity containing a blood-stained fluid. The rats died from tumors in the interval between the 30th and 39th days after inoculation.

Inoculation of Sarcoma M-1 into the Testicle

For inoculation of the sarcoma M-1 into the testicle 105 rats were used (Table 1).

In 28 animals the tumors in the testicles grew to some extent, increased perceptibly in size and then became necrotic, ulcerated and underwent complete regression. Usually in these rats the site of inoculation was fully cicatrized on the 30th-35th day from the start of the experiment.

TABLE 1

Results of Inoculation of the Rat Sarcoma M-1 into the Testicle

Series No. of experiments	No. of rats in each separate series of experiments	Tumors			Metastases	Day of death of rats from developing tumors
		inoculated	not inoculated	regressed		
		number of rats				
1	12	8	2	2	8	25—30 th
2	8	5	2	1	5	25—31 st
3	8	4	2	2	4	27—35 th
4	8	3	2	3	3	30—32 nd
5	9	4	—	5	4	29—36 th
6	16	2	8	6	2	31—35 th
7	8	5	1	2	5	25—31 st
8	10	7	1	2	7	26—39 th
9	10	8	—	2	8	21—30 th
10	8	7	—	1	7	22—28 th
11	8	6	—	2	6	25—33 rd
Total	105	59	18	28	59	21—39- th

In 18 rats the tumors did not grow in the testicles.

In 59 rats the tumors were successfully inoculated and on the 12th-17th day they could already be felt in the abdominal cavity of the animals in the form of dense, nodular formations. On pathological examination of these rats after slaughter or natural death tumor nodules of various sizes were found in the omentum, on the mesentery of the small and large intestine, on the peritoneum, the diaphragm and the surface of the liver and kidneys and in the thickness of muscle tissue situated far from the site of the original inoculation.

Not once did we observe the development of metastases directly in the lymph glands, the liver, kidneys, lungs, spleen or other internal organs. Thus, metastasis of the sarcoma M-1 differs somewhat from the development of metastases from, for example, the Brown-Pearce rabbit carcinoma, which metastasizes intensively in the internal organs of experimental animals.

Rats with developing tumors died during the period from the 21st to the 39th day from the start of the experiment. In Fig. 1 is shown an experimental rat (No. 334) in whose peritoneal cavity multiple metastases developed. It died on the 33rd day.

At necropsy the findings were as follows. There was a large tumor in the right testicle. In the omentum and mesentery of the small and large intestines was a continuous conglomeration of tumor tissue, the size of a

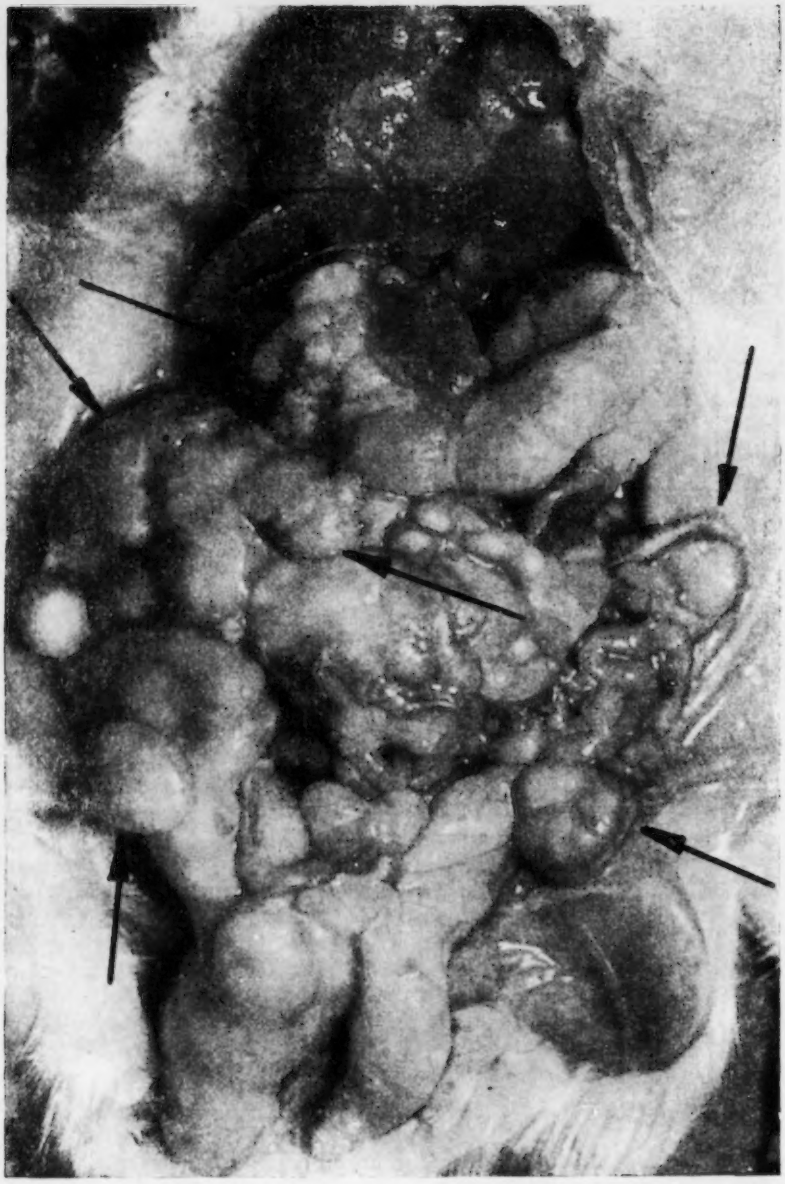

Fig. 1. Metastases of a sarcoma M-1 in a rat in the peritoneal cavity as a result of inoculation of the tumor into the testicle. The rat died on the 25th day from the moment of inoculation. The arrows indicate the situation of the metastases.

large hen's egg, completely isolated from the tumor developing in the site of inoculation. On the abdominal wall there were solitary tumor nodules of various sizes. In the region of the right kidney there was a large tumor nodule infiltrating muscle tissue. The kidney was slightly deformed and displaced. On the capsule of the left kidney were two metastases the size of peas. In the liver, kidneys, spleen, lymphatic glands and lungs no metastases were found.

TABLE 2

Distribution of Metastases from Sarcoma M-1 in Rats after Inoculation of the Tumor into the Testicle

Series No. of experiments	No. of rats giving metastases in each separate series of experiments	Metastases				
		in the omentum	on the peritoneum	on the diaphragm	in muscle tissue	on the surface of the other organs
		number of rats				
1	8	8	4	3	4	+2
2	5	5	1	4	3	—
3	4	4	1	1	1	—
4	3	3	—	—	—	—
5	4	4	—	2	1	1
6	2	2	—	1	—	—
7	5	5	2	2	3	—
8	7	7	5	3	1	1
9	8	8	1	2	5	—
10	7	7	4	3	3	—
11	6	6	1	3	2	—
Total	59	59	19	24	23	4

In Table 2 is shown the distribution of metastases from sarcoma M-1 in rats after implantation of the tumor in the testicle: in the omentum — 100% of the animals in which growth of the tumor took place, on the diaphragm — 40.6% of these animals, in muscle tissue — in 38.7%, on the peritoneum — in 32.2%, on the surface of organs (liver, kidneys) — in 6.7% of animals.

In order to make a comparative study of the biological properties of the "primary" (growing at the site of implantation under the skin) M-1 tumor and of its metastases, we carried out 5 series of experiments in which one group of animals was inoculated subcutaneously with tumor tissue from a metastasis in the diaphragm and another group of rats from the "primary" subcutaneous tumor nodule. According to the findings of E. E. Pogosiants [2], the latent period of growth of sarcoma M-1 when implanted under the skin varies from 5 to 7 days. The number of positive inoculations is 98-100%. According to L. M. Shabad, the number of positive inoculations under the skin is on the average 50%.

In our experiments, as may be seen in Table 3, the number of positive subcutaneous implantations in rats of the "primary" tumor was about 75% and the latent period of growth varied between 4 and 7 days.

The results of subcutaneous implantation in rats of tissue from a metastasis from sarcoma M-1 in the diaphragm were slightly different from those of the controls. The number of positive implantations in this case was about 32.1% and the latent period of growth varied between 8 and 12 days. Thus, during growth of the tumor in the diaphragm a weakening took place in the malignancy of the tumor tissue, which was reflected in the lower percentage of positive results of inoculation and in the lengthening of the latent period of growth.

There is no single opinion either on the morphological properties of the sarcoma M-1 growing in the subcutaneous cellular tissue. Thus, according to L. M. Shabad, sarcoma M-1 is mainly composed of spindle-shaped cells and is partly polymorphocellular. According to E. E. Pogosiants this tumor, histologically speaking, is a polymorphocellular sarcoma.

In our investigation the subcutaneous transplant of sarcoma M-1, as seen in Fig. 2, has a mixed structure: alongside polymorphous cells may be seen elongated cells and spindle-cell forms. In some places the cells are

Fig. 2. Histological section of a metastasis of sarcoma M-1 in the diaphragm. Stained with hematoxylin-eosin. Magnification 536 ×.

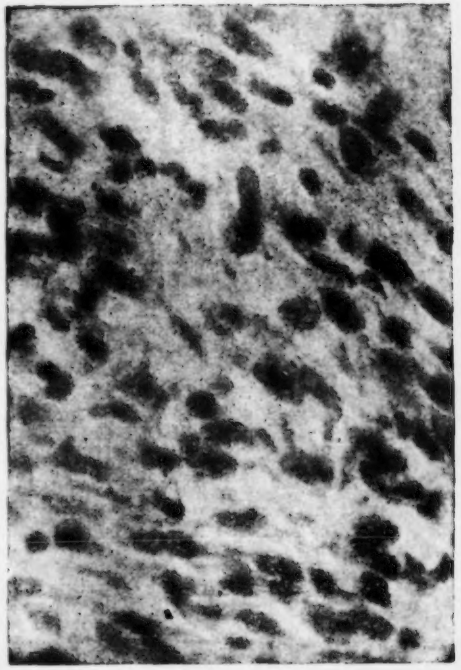

Fig. 3. Histological section of a subcutaneous transplant of sarcoma M-1. Stained with hematoxylin-eosin. Magnification 536 ×.

disposed in the same direction and form characteristic vortical structures. In the field of vision may be seen cells whose nuclei have a round or slightly elongated oval shape.

TABLE 3

Results of Subcutaneous Inoculation of Rats with Ordinary Tumor and with Tissue from a Metastasis of Sarcoma M-1 in the Diaphragm

Series No. of experiments	No. of rats in each experiment	Latent period of growth of the tumor (in days)	Results of implantation of tumor		
			positive	negative	temp. growth of tumor and subsequent regression
Experiment (inoculation of metastatic tissue)					
1	15	10—12	9	3	3
2	17	8—9	3	14	—
3	10	—	—	10	—
4	9	9—11	4	5	—
5	5	8—10	2	3	—
Total	56	8—12	18 (32.1%)	35 (62.5%)	3 (5.3%)
Control (inoculation of "primary" subcutaneous tumor)					
1	15	5—6	10	2	3
2	17	4—7	13	1	3
3	10	4—6	7	1	2
4	10	5—7	8	1	1
5	8	4—5	7	—	1
Total	60	4—7	45 (75%)	5 (8.3%)	10 (16.6%)

The histological structure of the tissue of the metastasis from sarcoma M-1 in the diaphragm is shown in Fig. 3. Here the cells are mainly polymorphous, with large nuclei. If spindle-cells are encountered, they are very few. The tissue of the metastasis is rich in stroma. Neither are the vortical structures in evidence, as in the subcutaneous transplant. The cells are arranged haphazardly.

The results described thus show that in consequence of changes in the conditions of existence, both the malignancy and the morphological structure of the M-1 sarcoma may undergo modification.

Our experiments confirm the findings of V. N. Popov that the M-1 sarcoma produces metastases when implanted in the testicle. However, in contrast to this worker, we did not once meet the development of metastases in the internal organs of the experimental animals, but observed them exclusively on the serous membranes of the organs.

SUMMARY

Sarcoma M-1, as a rule, produces no metastases when inoculated subcutaneously in rats. Metastases develop in the abdominal cavity of experimental animals when this tumor is inoculated into the testicle. Decrease of malignancy is noted in metastatic tumor on the surface of the diaphragm and is manifested in reduced percentage of positive inoculations and in prolonged latent period in subcutaneous inoculation of the tissue from the metastatic tumors in rats.

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CULTIVATION OF HUMAN LEUKEMIA FACTOR ON THE CHORIOALLANTOIC MEMBRANE OF CHICK EMBRYOS

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The majority of causative agents of virus diseases of man and animals possess a well-marked property of conservation and proliferation in the developing chick embryo.

The same results have been found in respect to a number of tumor viruses.

During the study of the nature of human leukemia factor,* the necessity for attempts at its cultivation on the membranes of fertilized chick embryos naturally arose.

In the present paper are described the results of investigations carried out in this field, under the direction of Active Member of the Academy of Medical Sciences of the USSR Prof. L. A. Zil'ber.

EXPERIMENTAL METHOD

The following were used for cultivation in chick embryos: fresh whole blood from 6 patients with acute leukemia; blood from 7 healthy persons (control); filtrate from the brain of a person dying from acute hemocytoblastosis; filtrate from the brain of a person dying from cardiovascular disease (control).

Cultivation was carried out by the usual method, as follows: 0.3 ml of blood (or brain filtrate) was placed in the intervascular region of the chorioallantoic membrane of a 7-day chick embryo of the Leghorn breed. The embryos were kept at 37°C. Five days later the allantoic fluid of the inoculated embryos was collected and injected (0.3 ml each) into fresh embryos.

Cultivation of the blood and brain filtrates from sick and healthy personswas carried out 7 times with different numbers of passages (4, 9, 10, 12, 15, 15, 40). The maximum number of passages was 40 and their general duration 6.5 months.

It has to be emphasized that the first inoculation of the embryos and all subsequent passages were made with the addition of 0.025 ml (0.625 mg) of cortisone to 0.3 ml of allantoic fluid. By adding cortisone we wished to suppress the organism-host reaction to the injected pathogenic agent.

For the mouse inoculations allantoic fluid was selected from the 3rd, 4th, 5th, 6th, 7th, 9th, 12th, 15th and 18th passages (for convenience in the table some experiments made with different numbers of passages from different patients are combined since no great variation was observed in their activity).

Animals were also injected with filtrates of the chorioallantoic membrane of the 7th and 9th passages of the blood from a patient with acute leukemia. To prepare the filtrate the membrane was finely chopped in cold sterile

* The term "human leukemia factor" conventionally describes an acellular agent isolated from human leukemia tissues [1].

Preparation	Age of mice used in the experiment	Mode of injection of preparation	Number of mice in the experiment	Time of appearance of 1st leukemia in mos.	No. of mice surviving until the appearance of the 1st leukemia	Mean latent period of development of leukemia in mos.	No. of leukemia developing	No. of leukemoid reactions	Remarks
Allantoic fluid of the 3rd–4th passages of leukemic blood	15 days	Subcutaneously	23	2.0	13	4.0	2	3	Transplantation in one case negative (myeloid reaction)
Allantoic fluid of the 6th passage of leukemic blood	2—8 days	Subcutaneously	83	1.0	46	1.3	10	19	Transplantation in 2 cases
	1 month	Into the spleen	21	2.0	4	4.0	2	4	successful (leukemia) and 1 case negative
Allantoic fluid of the 12th and 15th passages of leukemic blood	1—1½ months	Into the spleen	82	1.0	55	3.9	12	23	Transplantation in 2 cases successful (leukemia) and in 3 others – negative (reaction)
Filtrate of the chorioallantoic membrane of the chick embryo of the 7th and 9th passages of leukemic blood	11—30 days	Subcutaneously	29	2.0	14	2.0	1	2	
	1 month	Into the spleen	14	8.0	4	8.0	1	2	
Allantoic fluid of the 6th and 12th passages of leukemic brain filtrate	1—5 days	Subcutaneously	40	2.5	28	3.3	2	4	Transplantation in one case
	1—2 months	Into the spleen	40	1.5	12	2.7	4	6	successful (leukemia)
Allantoic fluid of the 6th and 7th passages of normal blood	2—8 days	Subcutaneously	33	—	28	—	0	2	
	1 month	Into the spleen	41	3.0	28	3.0	1	16	
Allantoic fluid of the 18th passage of normal blood	7—11 days	Subcutaneously	55	1.0	16	1.0	1	6	
Allantoic fluid of the 5th passage of filtrate of normal brain	1 month	Into the spleen	21	—	11	—	0	6	Transplantation in 1 case negative (reaction)
	6 days	Suboutaneously	21	—	13	—	0	2	

physiological saline (in proportions of 1 : 5) at pH 7.3-7.5. The suspension was centrifuged at 2000, 4000 and 6500 rpm. The precipitate was removed. The supernatant fluid remaining after the last centrifugation was passed through a Seitz filter. Films of the filtrate were examined under the phase-contrast microscope and also were stained by Pappenheim's method. Whole cells were absent from the films. Penicillin was added to the filtrate. The filtrates were sterile.

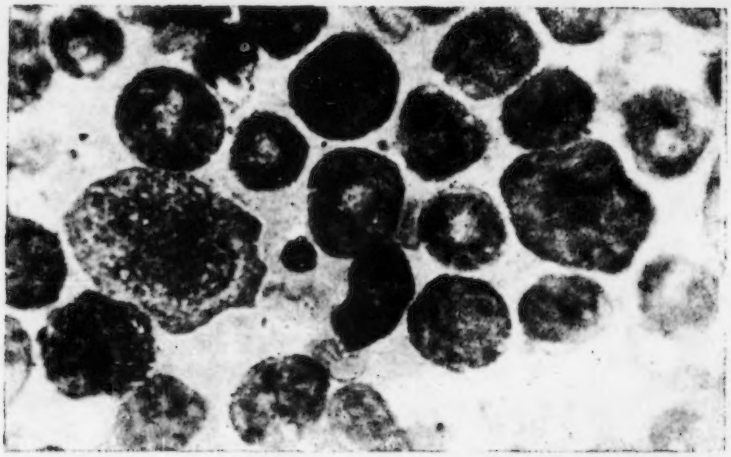

Fig. 1. Impression of the bone marrow. Myeloid leukemia. Magnification 720 ×.

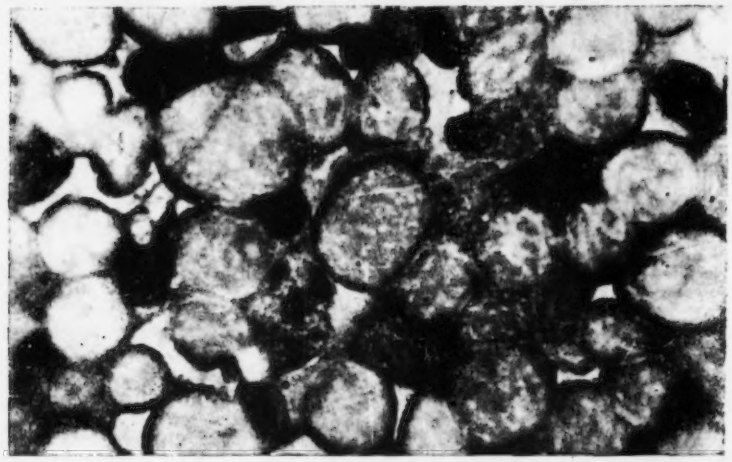

Fig. 2. Impression of the spleen. Leukemia. Magnification 720 ×.

Filtrates of human brain tissue were prepared in the following way. The tissue was macerated in the cold for 4 minutes in a homogenizer in a proportion of 1 : 5 with sterile physiological saline to which was added a 10% solution of 96° ethyl alcohol. The supension was kept at 4° for 20 hours and then centrifuged at 2500 rpm for 10 minutes. The supernatant fluid was passed through a No. 3 Rublevskii filter.

We judged the presence of human leukemia factor in the inoculated embryos by the result of a test of the specific biological activity in animals of the allantoic fluid taken from the embryos (the results of its specific antigenic properties were given in another paper [2].

The allantoic fluid and filtrates of chorionic membrane of the chick embryos were injected in a dose of

0.05-0.1 ml subcutaneously in newborn mice or directly into the spleen of adult mice of low-leukemic strains (CC57, C3, HA, C57 and white of no particular strain). Experiments were performed on 576 mice.

216 animals were examined histologically and cytologically.

For confirmation of the diagnosis of true leukemia in ambiguous cases transplantations of the resulting lesions into other mice of the same strain were carried out. It must be pointed out that attempts to transplant material from animals showing severe myeloid reactions (distinguishable from leukemias morphologically with difficulty) did not lead to the development of true leukemias.

Cultures of the blood of the experimental animals revealed no paratyphoid infection which may give leukemoid reactions in mice.

EXPERIMENTAL RESULTS AND DISCUSSION

The results of the experiments are summarized in the table.

As can be seen from the table, the allantoic fluid of the 3rd-4th, 6th, 12th and 15th passages of blood from patients with leukemia causes the appearance in mice of true leukemias in 20.5% of cases (28 leukemias in 136 mice surviving until the time of appearance of the first leukemias), their average latent period of development being 3.8 months. No essential variation in the activity of the allantoic fluid from different passages could be detected.

The allantoic fluid of the 6th, 7th, and 18th passages of blood from healthy persons led to the development of true leukemias in only 2 mice (of 72), which amounts to 2.7% of mice surviving until the time of appearance of the first leukemias.

The allantoic fluid of the 6th and 12th passages of leukemia brain filtrate, when injected into animals, caused the appearance of leukemias in 15% of mice (6 leukemias in 40 mice surviving until the time of appearance of the first leukemia) with a mean latent period of development of 3 months.

The allantoic fluid of the 5th passage of filtrate of normal brain did not produce leukemias.

It should be mentioned that the activity of the 12th passage of leukemic brain filtrate was slightly lower than that of the 6th passage.

The leukemias produced in the mice by their morphological structure belonged to the myeloid leukemias and reticuloses (Figs. 1 and 2).

So far as the leukemoid reactions (as a rule, myeloid) are concerned, they were observed equally often in the experimental and control groups.

We did not intend to make a systematic study of the lesions produced in the embryos. Nevertheless we must point out the severe retardation in development (growth), the massive hemorrhages in the organs and membranes, and the appearance of white jelly-like formations on the body of the embryos inoculated with blood from leukemic patients.

Thus, the results of the experiments described show that human leukemia factor is capable of prolonged (at least over 3 months) cultivation on the membranes of the developing chick embryo. However, judging by these experiments, its biological activity does not increase with passage.

There are several reports in the literature of the possibility of cultivation of noncellular agents isolated from animal tumors in chick embryos. These include: the agent of fibroma of rabbits [13], myxoma of rabbits [11], fowl leukemia [8], fowl lymphomatosis [9], leukemia of mice [10] and cancer of the mammary glands in mice [3, 4].

In chick embryos inoculated with noncellular agents from lymphogranulomatosis [6] and from chorionepithelioma in man [12] changes are observed which, in the authors' opinion, indicate the presence of filterable etiological agents in these tumors.

The research of A. D. Timofeevskii and his co-workers [5], who used electron microscopy and immunological reactions to identify the etiological agents of several tumors of man and animals in chick embryos, is of great interest.

The prolonged survival of the agent of human leukemia on the membranes of chick embryos, which we established by biological experiments and also by immunological reactions [2], enable it to be grouped with these etiological agents enumerated above.

Although the findings described in this paper are in support of the virus nature of human leukemia factor, the experiments of Brachet [7] must not be forgotten, for he showed the possibility of cultivation of lipoprotein cell fractions on the membranes of chick embryos (these experiments, it is true, are still disputable).

SUMMARY

Allantoic fluid of chick embryos, infected by blood and filtrate of human brain tissue from patients with leukemia or persons dead from it, was injected to mice of low-leukemic breeds. It was demonstrated that the above substances cause the appearance of leukemia in 20.5 and 15% of animals. Allantoic fluid of chick embryos infected with blood of healthy persons caused leukemia only in 2.7% of mice, while allantoic fluid of embryos, infected by filtrate of normal brain tissue did not possess leukosogenic activity. Consequently, it is possible to cultivate the human factor for a long period of time on membranes of developing chick embryos (15 inoculations for over 3 months).

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** In Russian.

THE CARCINOGENIC ACTIVITY OF THE MILK FACTOR AFTER STORAGE FOR 6 YEARS IN THE DRY STATE

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In previous papers on this problem [3, 4] it has been shown that the virus isolated from macerated tissue of a mammary gland tumor of mice together with the so-called nucleoprotein fraction (in short, the milk factor), when contained in an ampule sealed in vacuo at –70°C, does not lose its carcinogenic properties for 2 years if it is kept at +4°C. A method was also described for isolating the virus from the nucleoprotein components and of the further preparation required for its long storage.

In the present paper we give the results of two more experiments, in which two samples containing the nucleoprotein virus were tested after a far longer storage period in the dry state.

EXPERIMENTAL METHOD

One of the two samples of virus tested was opened on December 13, 1955, i.e., 5 years and 10 months from its date of preparation. The contents of the ampule were added to physiological saline and injected into 27 young female mice soon after birth – aged from 1-10 days (see table). The contents of another similar ampule were injected into 30 newborn female mice on February 13, 1956, i.e., 6 years from the date of preparation. Each ampule contained 286 mg of nucleoproteins, corresponding to 1075 mg of the original tumor tissue [3]; consequently, in the first experiment each mouse received 10.6 mg of nucleoproteins from 40 mg of original tumor tissue and in the second experiment each mouse received 9.5 mg of nucleoproteins from 35.8 mg of original tumor tissue. Just as in the two previous experiments, the material was injected as a single dose subcutaneously in the right groin directly into the next to the last mammary gland.

In both experiments the recipient mice belonged to the CC-57 white strain of the 30th inbred generation. Females of this strain do not develop carcinoma of the mammary glands [1, 2, 5, 6] but are susceptible to milk factor, and in experiments to test the carcinogenic activity of this factor, carried out according to a standard method with extract from recent tumors, on the average 50-55% of them developed carcinoma of the mammary glands [6].

On reaching the adult state the injected mice were removed from their mothers and marked according to a definite system; males were placed with them and remained with the females for not less than one year. Throughout the whole experiment the mice were examined once a month and any dying from any cause were autopsied, and their internal organs examined in detail. Data on the duration of survival of the experimental females and of the tumors arising in them are presented in the third and fourth lines of the table (IIIa, IIIb). Since the results of the tests of both samples are essentially the same, in the fifth line of the table they are given jointly for the two experiments.

In the first and second lines of the table (I, II) for the sake of convenience the results of previously published experiments (I and II) carried out with specimens of virus stored for 1 and 2 years respectively are given. In the

Experiment No.	Length of storage of virus	No. of mice injected	Dose per mouse (in mg): tissue	Dose per mouse (in mg): nucleoprotein fraction	No. of injections	Age of recipient mice: at expt. start (days)	Age of recipient mice: months 7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Tumors of the mammary glands produced: No.	Tumors of the mammary glands produced: %
I	1 year (3)	20	53	14.3	1	5—9	17	17	16	16	16	11	11	10	8	5	4	3	2						10	62.5
II	2 years (4)	27	40	10.6	1	5—17	26	26	26	25	24	20	17	13	12	11	7	6	4	2	2				4	15.4
IIIa	5 years 10 months . .	27	40	10.6	1	3—10	15	15	15	15	15	15	14	14	14	14	13	12	10	9	9	9	8	7	—	—
IIIb	6 years	30	35.8	9.5	1	1—9	17	17	17	17	17	17	17	16	15	14	10	10	10	8	8	7	7	6	1	—
	Total in expts. IIIa and IIIb	57	—	—	—	—	32	32	32	32	32	32	31	30	29	28	23	22	20	17	17	16	15	11	1	3.1
	Control (6)	—	—	—	—	—	17	7	10	1	13	14	13	8	1	7	24	23	4	2	1	3	—	—	—	—

sixth line are given the control results showing the absence of carcinoma of the mammary glands from mice of the CC-57 white strain between 1953 and 1957 [6].

The males are not included in the results of all these experiments since in these experimental conditions it would serve no useful purpose.

EXPERIMENTAL RESULTS

32 mice attained the "cancer" age for tumors of the mammary glands, i.e., 8 months. Later on the number of mice gradually fell. 22 females survived 18 months and a few lived for 2 years and more (see table).

Among this number of mice a mammary gland tumor developed in only one of the females in experiment IIIb, into which the test sample of virus was injected afterstorage for 6 years in the dry state. The tumor grew very slowly and at the end of the 3rd month of observation it was no bigger than a pea. Although testing of this tumor for the presence of milk factor is not yet completed, there are nevertheless grounds for believing that it developed under the influence of milk factor which still retained feeble activity in the sample of virus investigated. This is also shown indirectly by the fact that this particular tumor was one of a small number developing in CC-57 white mice in our other experiments devoted to testing the carcinogenic activity of tissue extracts from tumors of mice and man and they were completely absent from CC-57 white mice both in 1953-1957 and in the course of all the previous years [1, 2, 5, 6].

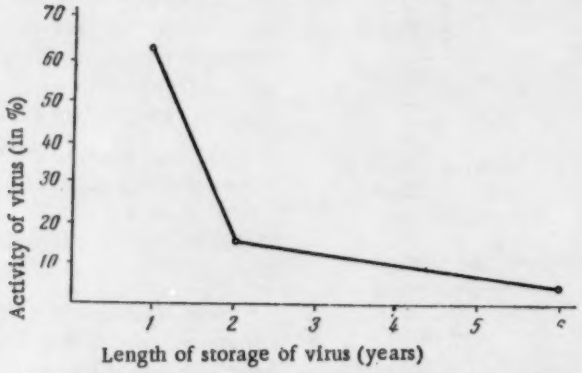
It should be mentioned that with increasing age of the tested virus and with a reduction in its biological activity tumors arose in older mice. This in experiment I (virus stored 1 year) the "cancer" age of the experimental mice varied from 9 to 19 months; in experiment II (virus stored 2 years) it varied from 12 to 16 months, and in experiment IIIb the only tumor of the mammary gland developed in a mouse 20 months after the injection of the virus.

In experiments I, II and III,besides carcinoma of the mammary gland,other tumors developed in other situations, in particular tumors of the lungs and of a few other organs. However the types and incidence of these tumors did not exceed the limits of their spontaneous development in CC-57 white mice .

The results of all three experiments thus show that the virus of carcinoma of the mammary glands under

The results of all three experiments thus show that the virus of carcinoma of the mammary glands under

these experimental conditions retains its carcinogenic activity for a comparatively long time. If the number of tumors in experiments I, II and III is related to the number of 8-month-old mice, i.e., at the "cancer" age for tumors in this situation, the percentage of mice with tumors in these experiments is 62.5, 15.4 and 3.1 respectively. The relation between the biological activity of the milk factor and the length of its storage period is shown graphically (see figure).



Graph of the relation between the carcinogenic activity of the virus (ordinate axis) and the length of its storage (abscissa) in the dry state.

The above values of the incidence of tumor formation are probably less than half their true value since it is known that tumors of the mammary glands arise on the average in only 50-55% of females of the CC-57 white strain of mice, even when the milk factor for the experiment is isolated from primary, i.e., spontaneous tumors or from recently excised transplanted tumors [6].

SUMMARY

The results of the conclusive experiment in the series concerning the blastomatogenic activity of the milk factor after a 6-year storage in the dry state are given. The experiments showed that the activity of the milk factor did not materially decrease after a one-year storage; however, it disappeared almost entirely when kept for six years the dry state.

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MORPHOLOGY AND PATHOMORPHOLOGY

THE RECEPTORS OF THE MAIN VEINS OF THE LIMBS IN DOGS AND MONKEYS*

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(Received August 9, 1957. Presented by Active Member of the AMN SSSR V. N. Chernigovskii)

Sensory nerve endings in the veins were first reported by A. S. Dogel' in 1898. He found nerve endings in the form of plates in the external coat of the veins of the heart. Reports have accumulated over a long period of time of the presence of receptors in various venous trunks [12, 22, 23] but descriptions of sensory nerve endings in the veins have usually been the result of fortuitous observations.

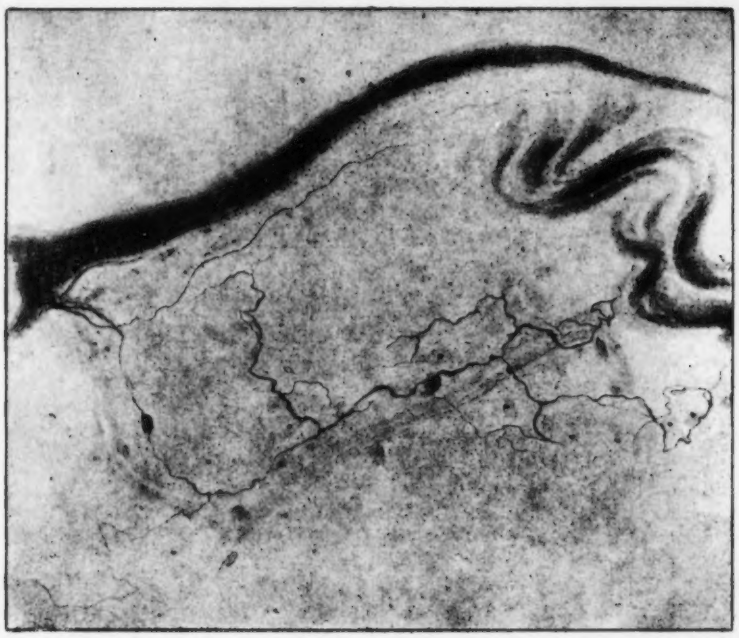

Fig. 1. The receptor apparatus in the adventitia of the wall of the great saphenous vein (in the upper third of the leg of an adult dog). Ramification of the receptor fiber among the network of vasa venarum. Bielschowsky--Gross impregnation. Magnification: ocular 10 X, objective 40 X.

* The substance of a dissertation submitted towards the degree of doctor of medical sciences.

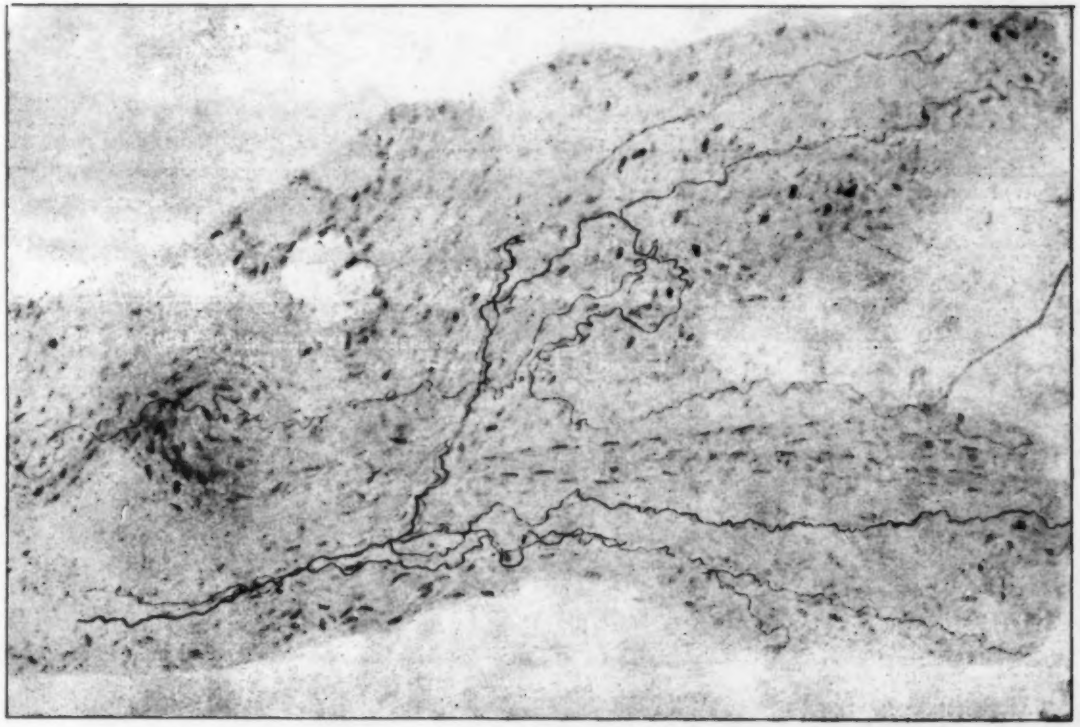

Fig. 2. The receptor apparatus in the adventitia of the cephalic vein (upper third of the arm of an adult dog). Bielschowsky-Gross silver impregnation. Magnification: ocular 10×, objective 40 ×.

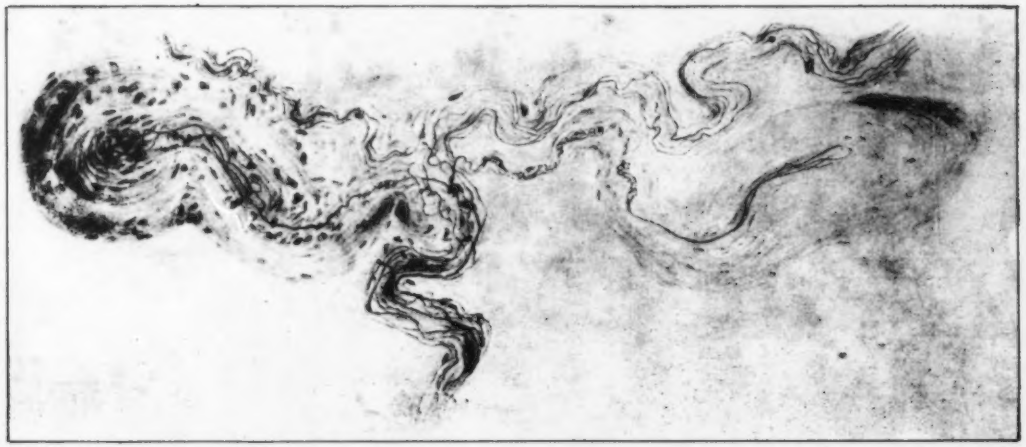

Fig. 3. One of the myelinated fibers of a nerve bundle in the adventitia of the brachial vein of the monkey (adult male Papio hamadryas). It is seen that the fiber divides into two branches, forming encapsulated endings of the Vater-Pacini corpuscle type. Bielschowsky-Gross impregnation. Magnification: ocular 10 ×, objective 40 ×.

Extensive research on this problem has been conducted by V. V. Astakova and his co-workers [1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 14, 15, 16, 17, 19, 20]. They did not confine themselves to describing the receptors under normal conditions of existence but they also studied the sources of origin of the nerve endings and their genesis, and the state of the nervous apparatus of the veins during clinical and experimental pathological conditions.

On the basis of numerous comprehensive investigations the presence of nerve endings was demonstrated in all the layers of the walls of the veins, including the endothelium. A double sensory innervation (bulbar and spinal) was shown by experimental means in the caval and pulmonary veins. The bulbar receptors were found to be more sensitive to all forms of action on the body (oxygen lack, experimental fever).

So far the problem of receptors in the walls of the veins of the limbs has received little attention; in the literature there are only isolated papers dealing with this subject, and the material is either incomplete [13] or quite unconvincing [18].

Our attention was directed to this problem in connection with the study of the collateral circulation developing as a result of resection of the main veins of the limbs. We investigated the nervous apparatus in the walls of the brachial, cephalic, femoral, and greater and lesser saphenous veins at various levels in 10 dogs and 6 monkeys (Papio hamadryas). The material was treated by the Bielschowsky-Gross silver impregnation method, the preparations being counterstained with hematoxylin. The sections varied in thickness between 30 and 90 μ.

As examination of the preparations obtained showed, in all the layers of the walls of the veins listed there are plexuses of unmyelinated and myelinated nerve fibers of medium caliber. The receptors formed by these belong mainly to the group of free nerve endings, with very extensive branching. Branches of the most diverse shapes are found — from simple antenna-like divisions of the nerve fiber to more complicated (Fig. 1) ramifications extending through many fields of vision (Fig. 2). More rarely it was possible to see encapsulated receptors of the Vater-Pacini corpuscle type, oblong in shape. In section it is possible to see clearly their external capsule and internal bulb. The nerve fiber at one pole of the corpuscle divides into several branches, terminating inside the bulb in pin-head thickenings. As seen in Fig. 3 a thin collateral emerges from the thick medullated fiber, proceeding with the thick fiber into the bulb.

We could find no endings with restricted ramification (de Castro's second type) in any of our preparations.

From an analysis of the receptor patterns it may be suggested that the main veins of the limbs receive their sensory innervation from the posterior roots of the spinal nerves. These endings, as we have seen, have usually an antenna-like shape and their branches extend over a wide area. B. A. Dolgo-Saburov and his co-workers established by a large number of observations and animal experiments that such receptors belong to the spinal group, i.e., they are formed from endings of the peripheral processes of the neurones of the spinal ganglia.

From a comparison of material obtained from dogs and monkeys no individual peculiarities could be distinguished. In the same way there were no differences in receptors in different veins (deep and superficial veins, veins of the fore- and hindlimbs).

SUMMARY

The results of investigation of the nervous apparatus in the walls of the brachial, cephalic, femoral, small and large saphenous veins, examined on various levels, are presented. 10 dogs and 6 monkeys (Papio hamadryas) were used for this experiment. The specimens were silver impregnated by the Bielschowsky-Gross' method. The presence of a network of nerve fibers of average size was found in all these veins. Receptors, formed by them, belong mainly to the group of free nerve endings. The latter are found in various forms from a simple branching of the nerve fiber to more complicated ramifications which spread to many fields of vision. Encapsulated receptors of Vater-Pacini type were more scarce. These receptors, as was established by B. A. Dolgo-Saburov and his collaborators on the basis of a considerable number of observations and experiments on animals, are related to the spinal group, i.e., they are formed by the terminations of the peripheral neurons of the spinal ganglia.

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THE NERVE SUPPLY OF THE DUCTUS ARTERIOSUS IN DOGS

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In their studies of the ductus arteriosus many workers have attempted to find the reason for its obliteration in the peculiar structure of the wall of the vessel [6, 11 and others] or in the topographical anatomical changes in the thoracic organs taking place after birth [7, 10]. The role of the nervous system in the function and obliteration of the duct has not been determined, and it would not be incorrect to say that it has been inadequately investigated. The exception is the work of Barcroft, Kennedy and Mason [4], who used physiological methods without preliminary anatomical investigation in an attempt to find nerves which brought about the closure of the ductus. By stimulation of the peripheral end of the left vagus nerve they succeeded in establishing a diminished blood flow through the ductus and, as assumed by these authors, its closure. They conclude from these findings that in guinea pigs the branches of the left vagus nerve must participate in the innervation of the ductus. However, more recent experiments by Kennedy and Clark [Clark, 1942] showed that destruction of the spinal cord and sympathetic ganglia with division of both vagus nerves, and also electrical stimulation of the vagus, phrenic, sympathetic and splanchnic nerves and of the stellate ganglion on the left side have no effect on closure of the ductus. The negative results of these experiments led these authors to the incorrect conclusion that the nervous system is not concerned in closure and function of the ductus. This problem is till unsolved at the present time even though a considerable amount of evidence has accumulated in the literature indirectly supporting the existence of receptor apparatus in the wall of the ductus arteriosus. Osseladore, Pezzuoli and Pietri [8], for instance, observed closure of the ductus arteriosus in guinea pigs immediately after injection into their blood vessels of a perfusing fluid saturated with oxygen, and relaxation of the ductus when the concentration of carbon dioxide in the blood was increased. Record and McKeown [9] observed constriction of the walls of the ductus when physiological saline was injected into the blood vessels of the animals.

T. A. Grigor'eva [1], studying the innervation of the vessels of the cat, discovered a large number of nerve cells in the region of the aortic orifice of the ductus arteriosus which, evidently, belonged to the vagus nerve. Similar observations were made by Boyd [5] and Jager and Wollenman [12].

The incomplete and contradictory accounts in the literature of the nerves of the ductus arteriosus impelled us, at Prof. B. M. Sokolov's suggestion, to carry out an experimental morphological investigation of the nerves of the ductus arteriosus in animals and especially in dogs.

EXPERIMENTAL RESULTS

The results of macro- and microscopic examination of the nerves of the ductus in 20 cadavers of puppies and fully-grown dogs showed that the nerves to the ductus arteriosus are formed from branches of the sympathetic trunk and the vagus nerve on the left side. In the course of the nerves to the ductus two forms can be distinguished – scattered, and combined in one main trunk. In the latter case ($^4/_5$ of all animals) the independent nerve to the ductus areteriosus (Fig. 1) is formed from branches of the left vagosympathetic trunk, the caudal cervical ganglion and the trunk of the left vagus nerve. The number of branches of the vagosympathetic trunk and the caudal cervical ganglion taking part in the formation of this nerve varies from 1 to 3, their thickness from 0.1-0.3 mm and their length up to 1.5 cm. The number of branches of the left vagus nerve is inconstant and varies from 1 to 4, their thickness from 0.1-0.3 mm, and their length up to 0.7 cm.

The branches are united into a nerve from the level of the VIth cervical to the IIIrd thoracic vertebra. In one case at the site of union of the branches we observed gangliar thickenings, oval in shape and measuring 1 × × 2 mm (Fig. 1).

Fig. 1. Formationof the nerve of the ductus arteriosus in a two-day-old puppy: vagosympathetic trunk and left carotid artery (2), innominate artery (4), caudal cervical ganglion (9), nerve of the ductus arteriosus (8), ductus arteriosus (10).

Fig. 2. The nerve of the ductus arteriosus in a two-month-old puppy: arch of the aorta (1), innominate artery (2), carotid artery (3), subclavian artery (4), pulmonary artery (5), nerve of the ductus arteriosus (7), branch to the arch of the aorta (9).

The nerve thus formed is 0.75 mm in thickness and up to 2.2 cm in length and it usually proceeds alone ($^2/_3$ of cases), situated on the anterior surface of the aortic arch from the orifice of the left subclavian artery to the medial border of the aortic orifice of the ductus arteriosus. More rarely the nerve has connections with the cardiac branches of the left vagus nerve taking part in the formation of the left coronary cardiac plexus (1 case in 20), with the trunk of the left vagus nerve and with the deep part of the cardiac plexus (2 cases in 20). Two variants can be distinguished in the distribution of the nerve to the walls of the ductus.

In the first variant the nerve divides when still 0.2-0.5 cm from the ductus arteriosus into branches 0.1-0.2 mm in thickness and up to 1 cm in length which, uniting with each other, form anastomoses in the form of a loop (Fig. 2).

In the second variant (Fig. 1) the nerve proceeds alone to the ductus, and only after becoming completely buried in the adventitia does it divide into branches directed toward its orifices.

In $^1/_5$ of cases where the nerve consists of a single trunk, the nerve of the ductus arteriosus arises not from the combination of branches but directly from the left caudal cervical sympathetic ganglion, and it has a thickness of 0.3-0.5 mm and a length of 1.5-4.5 cm. The nerve is distributed not only to the walls of the ductus but also to the cellular tissue lying on the anterior surface of the arch of the aorta in direct proximity to the aortic orifice of the ductus arteriosus. These branches were shown particularly clearly in fully grown dogs with an obliterated ductus. Inindividual cases, in its course toward the ductus the nerve has connections in the form of trunks 0.1 mm

in thickness and up to 2 cm long, with the nerves of the thymus gland, the pericardium and with the cardiac branches.

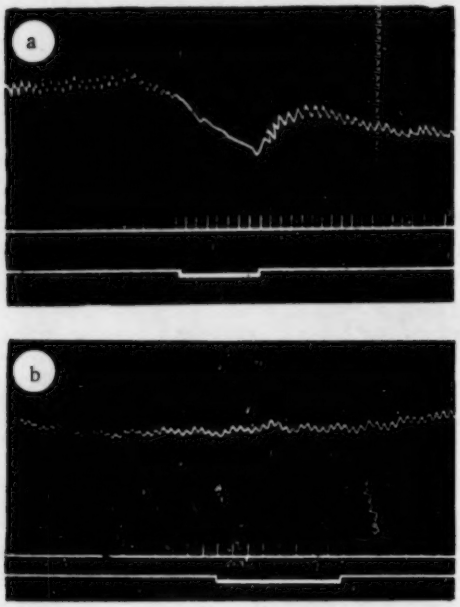

Fig. 3. Changes in the blood pressure of a two-day-old puppy during stimulation of the aortic orifice of the ductus arteriosus (a) and during stimulation of areas of the aortic adjacent to the orifice of the ductus arteriosus (b).

When the branches to the ductus assume the scattered form they appear as 2-5 nerve trunks emerging from the left caudal cervical ganglion and the left vagus nerve. The thickness of the branches of the ganglion is 0.2-0.5 mm and their length 2-4.5 cm; the thickness of the branches of the vagus nerve is 0.1-0.2 mm and their length up to 1.2 cm. In their course to the ductus arteriosus the branches always cross the anterior surface of the arch of the aorta. Before burying themselves in the wall of the ductus, as a rule, they have connections between themselves. The largest branches leading to the ductus arteriosus in this scattered form are the nerve trunks which emerge from the caudal cervical ganglion.

The main nerve trunk leading to the ductus arteriosus is usually situated in the area between the origins of the innominate and the left subclavian arteries, among the loose cellular tissue, and is directed inferiorly and caudally. Its constancy, its topographical anatomical characteristics and its distribution in the wall of the ductus arteriosus justify its being called the nerve of the ductus arteriosus (Figs. 1, 2).

Besides the above-mentioned nerves, branches from the left augmentor nerve of I. P. Pavlov, studied in detail by I. M. Shvetsov [3], enter the wall of the ductus. These branches are 0.2 mm in thickness and are up to 1 cm in length, and are distributed to the middle part of the ductus arteriosus and to its pulmonary aperture.

A concentration of nerve branches may be observed in the wall of the ductus in the region of its aortic end, which is in agreement with the findings of Boyd, Wollenman, Barcroft, T. A. Grigor'eva and others.

Experiments carried out jointly with A. P. Dvoinina on 20 dogs, mainly puppies (from new-born to $2\frac{1}{2}$ months of age), showed that the ductus arteriosus is a well-marked reflexogenic zone. The reflex is observed particularly clearly (fall in blood pressure in the greater circulation, slowing of the rate of the heart with intensification of the pulse pressure) during stimulation of the aortic orifice of the ductus (Fig. 3). We observed a similar reaction on stimulation of the nerve of the ductus arteriosus. Division of the nerve and subsequent stimulation of its central and peripheral ends demonstrated convincingly that this nerve is centripetal, since a depressor reaction is observed only on stimulation of its central end. As a control of these findings we stimulated the aorta adjacent to the ductus arteriosus, but we did not once observe a reaction of this sort in either puppies or fully grown dogs.

It is neccesary to point out that the reflex from the ductus arteriosus alters with age. It is particularly well marked in puppies in the first 5 days of life and is completely absent in adult dogs. Perhaps the anatomical changes in the ductus arteriosus (obliteration of the ductus and its exclusion thereby from the circulatory system) are connected with the age changes in the function of its nerves. It is possible that previous workers (I. Cion, K. Ludwig and others) who made extensive studies of the circulatory reflexes used for this purpose fully grown dogs and did not find the nerve of the ductus arteriosus, whose depressor function is timed to be shown in the first period of postembryonic development.

SUMMARY

The nerves of ductus arteriosus up to the present time were studied inadequately. The author performed experiments on 20 adult dogs and newborn pups in which he investigated the nerves of the ductus arteriosus by macro- and microscopic examination and physiological methods.

The results of these investigations demonstrated that the nerves passing in the direction of the ductus are formed from the branches of the truncus symphathicus and the vagus nerve of the left side. 2 variations of nerve distribution were found: scattered and magistral. In the last variant an individual nerve (the nerve of ductus arteriosus) approaches the duct. Its diameter reaches 0.75 mm in the newborn pups.

Experiments showed that the nerve of ductus arteriosus is a depressor. Its function changes with the age of dogs. It is especially pronounced in puppies during the first 5 days of life and is completely absent in adult dogs. The depressive function of the nerve of ductus arteriosus is meant to serve during the first period of postembryonic development when the depressive function of Ludwig-Cion's nerve is absent.

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CHANGES IN THE INTIMA OF VEINS FOLLOWING CHEMICAL ACTION ON THEIR WALLS

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During the last ten years new information has been obtained on the tissue properties of the endothelium of the vascular system in the vertebrate animals and man [1-13]. However, there still remain many unsolved and doubtful problems in this field.

The present investigation was devoted to the study of changes in the endothelial lining of the jugular veins of rabbits after the action of crystals and concentrated solutions of sodium chloride on their walls.

EXPERIMENTAL METHOD

In some experiments the wall of the jugular vein was exposed under aseptic conditions, and then a small crystal of sodium chloride was applied to the outside of the exposed area for 5-6 minutes. In other experiments

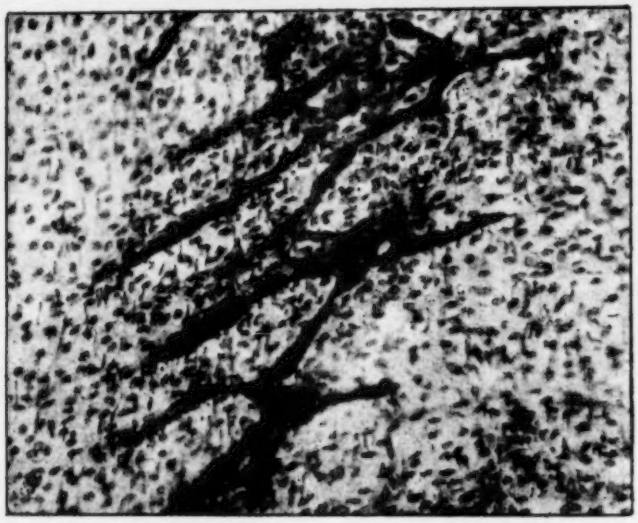

Fig. 1. Internal surface of the jugular vein of a rabbit 4 days after trauma. Multiple proliferations of the intima in the region of application of a crystal of sodium chloride. Total plane preparation. Iasvoin's hematoxylin stain. Microphotograph. Magnification: objective 20 ×, ocular 7 ×.

a solution of 20% sodium chloride was injected from a syringe into the external coat of the vein, in a dose of about 0.1 ml, and in a short time this infiltrated through the outer coat and partly through the muscular coat. In each case the operation wounds were closed in layers. The animals were killed on the 2nd-10th day, the damaged vessels were resected and incised longitudinally along the side opposite the point of action of the salt. After fixation in alcohol, total preparationswere made from the vessels, and stained with hematoxylin by Iasvoin's method, with preliminary impregnation of the cell margins of the endothelium with a 0.25% solution of silver nitrate or without this addition. Altogether 18 preparations of jugular veins were examined.

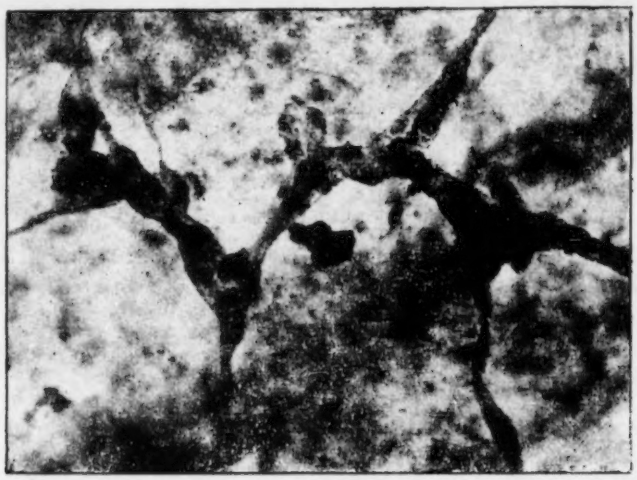

Fig. 2. Endothelial proliferation in the lumen of the jugular vein of a rabbit 6 days after injection of sodium chloride solution into the wall. Total plane preparation. Iasvoin's hematoxylin stain. Microphotograph. magnification: objective 40 ×, ocular 10 ×.

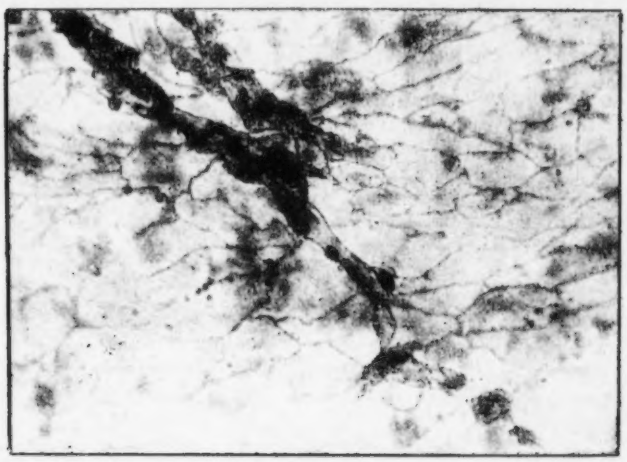

Fig. 3. Internal surface of the jugular vein of the rabbit 10 days after injection of sodium chloride solution into its wall. Transformation of an endothelial projection in the vein into an endothelial intravascular proliferation. Plane preparation. Margins of the endothelium impregnated with silver, Iasvoin's hematoxylin stain. Microphotograph. Magnification: objective 40 ×, ocular 10 ×.

EXPERIMENTAL RESULTS

During examination of the preparations in which it was possible to trace not only the initial coat throughout its full extent but also all the other coats of the vein walls, considerable proliferation of the intima into the lumen of the vessel was observed in nearly all the damaged veins. These began to appear on the 2nd-3rd day after the experimental chemical action and were very clearly marked after 4-10 days. The character of the proliferations was similar whatever the mode of trauma, and so they will be described together; in relation to the intensity of the lesion of the intima and of its area there are appreciable differences. After the action of a crystal of sodium chloride on the outer coat of the vein a considerable larger part of the intimal coat was affected than after injection of the salt solution into its wall. In the first case the dimensions of the proliferation were greater and their number larger than in the second case. This may be due to the fact that at the site of application of the crystal a very concentrated saline solution is formed.

The proliferations of the intima are in the form of single or multiple bands and threads which are joined at one, or – as is observed much more often – two ends to the intima (Figs. 1, 2, and 3). They are to a large extent freely disposed in the lumen of the vessel and are bathed in blood. Often the bands are interconnected and form a complex binding (see Fig. 1). In other cases in a group of proliferations there is one band which is thicker than the others, usually centrally situated, and joined by several thinner septa to the intima.

The majority of the proliferations consist of endothelium only, and their thickness is very insignificant (see Figs. 2 and 3); others, which are thicker, are composed of fibrous connective tissue covered with endothelium. The purely endothelial proliferations (see Figs. 2 and 3), which are derived from the initial endothelium of the vein, are of the greatest interest. In preparations in which the margins of the endothelial cells are impregnated with silver, the transformation of the endothelium of the wall of the vein into the endothelium of the bands extending into the lumen of the vessel (see Fig. 3) is clearly seen. In proliferations with a fibrous connective tissue basis, its connection with the connective tissue of the intima can readily be traced. On the 2nd-4th day after the chemical action of the vessel numerous mitoses can be seen in the cells of the endothelial lining. These facts provide one more confirmation of the ability of the endothelium, even of major vessels such as the jugular veins of the rabbit, to proliferate actively in response to trauma and to give rise to intravascular proliferations.

It was possible to trace the method of formation of these proliferations. In some places the surface of the intima becomes uneven on the 2nd-5th day and the endothelium forms either single or multiple projections which then increase in length although not beyond the bounds of microscopic dimensions. These projections are pin-head or dome-shaped. In some of the traumatized veins they attain a considerable length and hang in the lumen of the vessel in the direction of the blood flow. The very long and thick bands seen in the carotid and femoral arteries [6, 8] and in the aorta [1, 2] could not be observed. The possibility is not excluded that they may become very long in the veins too, then become attached by their free end to the intima of the vessel, forming bands fixed by both ends to the vessel wall.

In other cases thickenings are found in the intima of the vein in the form of rods directed with the blood flow. Subsequently the middle portion of a rod, consisting of connective tissue covered with endothelium becomes separated from the vessel wall and forms a bridge, freely disposed in the lumen of the vessel and connected to its wall by its ends. This method of formation of intravascular proliferations of the intima is clearly traceable in histological preparations.

On the internal surface of some vessels appear, in addition to the proliferations mentioned above, funnel--like craters, lined with endothelium. They are not deep and terminate blindly. It may be considered that these formations arise in consequence of infiltration of the endothelium into the thickness of the vessel wall. They are probably the initial stages of development of new intramural vessels similar to those described elsewhere [1-3, 6-8, 13].

The results obtained show that stimulation of the wall of a vein with chemical substances with a relatively low traumatic power causes proliferation of the intima which alters the hemodynamics in this section of the vessel. They lead to interference with the blood flow and to stasis, as a result of which favorable conditions are created for partial or complete thrombosis of the vessel. These findings confirm once again the necessity of using harmless operative agents during various procedures on the vessels in order to avoid undesirable late consequences.

From a comparison of the findings described in this paper with those in the literature it is impossible to escape from the conclusion that during trauma to the wall of a blood vessel there is regularly found a biological

reaction of the endothelium, as shown by the active mitotic growth of this tissue and the formation of intravascular and intramural endothelial proliferations.

SUMMARY

An active mitotic proliferation of endothelium was found in rabbits following the action of sodium chloride crystals or 20% solution of sodium chloride on the outer coat of the jugular veins. Later, the endothelial outgrowths were revealed both in the veins and within their walls.

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THE EXPERIMENTAL FORMATION OF DECIDUAL TISSUE

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The problem of the genesis of decidual tissue is of considerable complexity and is still largely unsolved, particularly with regard to the fact that the decidual reaction is found not only in the uterus but also occasionally elsewhere, for example, in the ovary, pelvic peritoneum, omentum, appendix and so on. Several workers have published in the literature [1, 3, 4] reports of endometrioid heterotopia and of ectopic decidual tissue. K. P. Ulezko-Stroganova [4] attaches great importance to hormonal and metabolic stimulation and at the same time considers that the development of endometrioid heterotopia may also be connected with the effect of a persistent inflammatory condition. V. S. Lisovtskii [2] considers that the development of decidual tissue is due to the hormonal effect of the corpus luteum and the growing ovum, inflammation being secondary.

Our concern in this problem derives from the results of our experiments on nonpregnant guinea pigs in which the phenomenon of hyperergasia had been produced in the uterus, where the development of decidual tissue was observed after an assaulting injection. The results of these experiments, carried out during a state of allergy, make us critical of certain statements of Loeb [7-9], and enable us to add new facts and to define certain points more precisely, which were incorrectly treated in the work of Loeb.

As a result of many experiments carried out in 1907-1910, Loeb came to the conclusion that the development of decidual tissue is possible without the participation of the ovum whose role is only that of a mechanical factor, providing a stimulus for the decidual conversion of the endometrium. The effect of the ovum can be replaced by other mechanical stimuli, for example an incision of the uterine wall, the injection of a foreign body into its cavity and so on. 24 hours after the application of the trauma proliferation of the interglandular tissue of the endometrium, may be found, and on the third day many mitoses and enlargement of the cells of the stroma, mainly beneath the integumentary epithelium. During 5-9 days after incision of the uterus may be observed the formation of small decidual nodules, which attain their full development on the 10th-11th day; after the 15th day the deciduum undergoes necrosis.

Loeb asserts that the decidual transformation of the endometrium under the influence of mechanical stimulation, just as after the action of the ovum itself, is possible only where there has been preliminary sensitization of the uterus to the hormones of the corpus luteum. This sensitization takes place from the 3rd to the 9th day after coitus. Loeb considers that the same interval (4-9 days) after menstruation is optimal for the development of decidual tissue without coitus. Since corpus luteum hormone is necessary not only for sensitization of the uterus but also for the life of the decidual tissue, regression of the corpus luteum and disappearance of its internal secretion leads to necrosis of the deciduum.

The results quoted were obtained by Loeb in nonsensitized guinea pigs. In our own experiments the guinea pigs were sensitized to horse serum and the development of decidual tissue took place in association with an allergic state. Loeb used for a stimulus incision or puncture of the uterus, and so on. Our stimulus was combined and consisted of injection of horse serum into the uterus supplemented by cooling of the experimental horn with ethyl chloride for 3 seconds or by the addition of ergotin to the serum. It is important to mention that the decidual reaction appears when the assaulting stimulus is applied at a particular stage of the sexual cycle – the

resting stage (dioestrum). Outside this stage the application of such a stimulus to the uterus resulted in a violent inflammatory reaction with formation of superficial and deep ulcers in the endometrium.

We give a short account of some of the experiments and of the histological findings.

EXPERIMENTAL METHOD AND RESULTS

Guinea pig No. 73, weight 456 g. Sensitization with 3 ml of horse serum 5 times at intervals of 6-7 days, starting on February 20. On March 25 vaginal smears showed the dioestrous stage. Laparotomy performed on the same day, with injection of 0.6 ml of serum into the right horn of the uterus. Immediately afterwards a stream of ethyl chloride is directed on to this horn for 3 seconds at a distance of 25-30 cm. The guinea pig was killed 4 hours after the assaulting stimulus. The right horn is thickened and hyperemic, with hemorrhages in the serosa.

Histological investigation. In places the uterine mucosa is more or less thickened, infiltrated with inflammatory elements and edematous. In the subepithelial zone there is irregular proliferation of the stroma which is undergoing a decidual metamorphosis. The mucosa here is poor in glands and mitoses are seen in the interstitial cells. Chorionic villi were not found. In the endometrium and circular musculature in places, areas of necrosis could be seen.

Guinea pig No. 47, weight 420 g. Sensitization and experimental procedure as in the previous example. Stage of the sexual cycle – dioestrum. Animal killed 48 hours after assaulting injection of 0.6 ml of horse serum into the left horn of the uterus, followed by cooling with ethyl chloride for 3 seconds at a distance of 25-30 cm.

Histological investigation. Under the integumentary epithelium is seen a decidual transformation of the stroma, the cells of which become round in shape and increased in size. There are many mitotic figures and few glands here. In places the mucosa forms polypi of decidual cells, among which mitoses are clearly visible. In some preparations an isolated islet of tissue, a transverse section through the apical portion of a decidual polypus, can be seen in the cavity of the cornu. Some of the vessels are dilated and filled with blood. Chorionic villi are not found.

Guinea pig No. 76, weight 478 g. Sensitization and conduct of the experiment as in guinea pig No. 73. Stage of the sexual cycle – dioestrum. Animal killed 72 hours after assaulting injection of 0.6 ml of horse serum into the left horn and the simultaneous action on this structure of a stream of ethyl chloride for 3 seconds from a distance of 25-30 cm.

Histological investigation. Part of the mucosa of the left horn is more or less thickened and consists, as it were, of two layers. The first – subepithelial, compact – is formed of decidual cells among which mitoses are seen Glands are few or completely absent. The second layer – beneath the first – is in direct contact with the circular musculature and contains a comparatively large number of glands. The stroma is infiltrated with eosinophils. In one place the mucosa forms a decidual polypus. Chorionic villi nowhere to be seen.

Guinea pig No. 51, weight 488 g. Sensitization and experimental procedure as in guinea pig No. 73. Stage of the sexual cycle – dioestrum. Animal killed 80 hours after assaulting injection into the left horn of 0.6 ml of horse serum mixed with one drop of a 10% solution of ergotin.

Histological investigation. In the superficial zone of the endometrium is observed proliferation of interstitial tissue, which in the caudal section of the horn forms a polypus of decidual cells, covered at the apex with prismatic, and elsewhere with elongated, simple squamous epithelium. In the central portion of the polypus a barely perceptible hyaline droplet degeneration of the decidual cells may be observed. At the base of the polypus is a small hemorrhage.

Guinea pig No. 53, weight 375 g. Sensitization and experimental procedure as in guinea pig No. 73. Stage of the sexual cycle – dioestrum. Animal killed 96 hours after assaulting injection of 0.6 ml of horse serum into the left horn and the simultaneous application of a stream of ethyl chloride for 3 seconds from a distance of 25-30 cm.

Histological investigation. The superficial layer of the mucosa consists of decidual tissue forming decidual processes into the cavity of the horn which is thereby diminished in size and in places is obliterated. In the peripheral zone of the polypi there are many mitoses but no glands. The cells of the central zone are enlarged and their large nuclei are oval or round in shape. The capillaries in this part of the polypus are compressed and hyaline droplet degeneration of the decidual cells is observed. Their protoplasm has disintegrated into a multitude of large and small homogeneous blocks, red in color. The nuclei of many of the cells have become pale and reduced in size. As a result of the penetration into this region of segmented neutrophil leucocytes some liquefaction of the

tissue takes place with the formation of cavities filled with a rose-colored fluid containing white blood cells and remnants of disintegrating cells. Outside the zones of cytolysis no leucocytes are visible. Chorionic villi are not seen. The basal layer of the endometrium consists of ordinary stroma with a small number of compressed glands.

From these descriptions it can be seen that in allergic conditions, after the application of a stimulus in the stage of dioestrum the rate of decidual transformation is speeded up. In guinea pigs Nos. 7, 24 and 73, for example, the beginning of the decidual reaction is already visible 4-6 hours after the assaulting injection. Beneath the integumentary epithelium of the endometrium proliferation of the stroma and the appearance of mitoses among its cells are observed. Later on the number of mitoses increases, the glands in this zone disappear and on the 3rd day polypi of decidual cells are observed (guinea pigs Nos. 43, 47, 76 etc.). By comparison with the periphery the cells of the central part of the decidual polypi are increased in size. In guinea pigs Nos. 51, 42 and 53, after 80, 91 and 96 hours respectively from the assaulting injection, hyaline droplet degeneration of decidual cells is found in the central part of the decidual mass, with subsequent liquefaction of these cells as a result of the enzymic activity of leucocytes which penetrate the area.

Thus, our results bring new facts to the problem of the times of development and involution of the decidua. The beginning of the decidual transformation of the endometrium, for example, was already marked in our experiments 4 hours after the assaulting injection, while in Loeb's series this time was lengthened to 24 hours. We observed the appearance of a formed decidua on the 3rd day, i.e., 8 days before Loeb. Finally, the beginning of regression of the decidua, in the form of hyaline droplet degeneration and cytolysis was found in our experiments on the 4th day, whereas in Loeb's experiments involution of the decidua began 13-15 days after trauma.

In Loeb's opinion the development of decidual tissue is influenced only by mechanical stimulation of the endometrium previously sensitized with corpus luteum hormone. The question naturally arises: under the influence of what impulses does development of decidual tissue take place during extrauterine pregnancy, when the endometrium is not subjected to mechanical stimulation from the ovum, which is arrested in some extrauterine position? It is quite obvious that mechanical stimulation of the mucosa of the uterus or Fallopian tube causes not only damage of the tissue but also stimulation of receptors. An assaulting action on the nerve structures is also shown by the absorbtion of products of disintegration resulting from mechanical trauma to the mucosa, whether due to artificial means (incision of the uterus) or to the ovum.

Afferent signals arising at the site of the trauma are effected by means of impulses which pass through the central nervous system along efferent paths directly to the ovary, or by a combined-reflex route, through intensification of the hormonopoietic function of the pituitary, or else by a short pathway directly to the uterus, stimulating the proliferation and decidual transformation of the endometrium previously sensitized with corpus luteum hormone. It is quite possible that this mechanism takes place during the development of decidual tissue in the uterus during extrauterine pregnancy and during decidual transformation of endometrial heterotopias.

The experiments of I. A. Eskin [5, 6] showed that if rats were injected subcutaneously for 3 days (on the day of estrus and the following two days) with a-folliculin in a dose of 4000-6000 international units, dioestrum is produced and lasts for 13.1 days on the average. On the 6th day after the injection of folliculin the uterus of three of the animals was sewn through with thread and in two of these a placentoma was found 5 days later. I. A. Eskin points out that the state of dioestrum caused by injection of folliculin is similar, both in its duration and in the decidual metamorphosis of the endometrium, to the normal pseudopregnancy of the rabbit and is accompanied by an active lutein phase in the ovary.

If a prolonged dioestrous state similar to that in pseudopregnancy is obtained as a result of appropriate stimulation of the nervous system (injection of folliculin or adrenalin, mechanical or electrical stimulation of the cervix of the uterus), it can be assumed that a similar phenomenon took place in our experiments also. This means that in our experiments the act of sensitization must be regarded as a stimulus of the nervous system. Probably the preparatory injection of serum, like the injection of folliculin in I. A. Eskin's experiments, coincided in some of our animals with the time of oestrus, and caused in these animals a prolonged dioestrum with activation of the corpora lutea arising during natural ovulation. With such an assumption it is not difficult to explain why the assaulting injection results in the formation of decidual tissue.

So far as the shortening of the periods of development and involution of the decidual tissue are concerned, the former, obviously, is connected with stimulation and the latter with the subsequent depression of nervous activity. Because of this the trophic influence of the nervous system during a state of allergy and in the absence of the ovum

is modified and is expressed early, presumably through reflex suppression of the function of the corpus luteum, in the decidual tissue which undergoes rapid regression. This type of mechanism probably lies at the root of some cases of early interruption of pregnancy and incomplete abortion in women who are of an allergic disposition.

SUMMARY

Nonpregnant guinea pigs were sensitized by horse serum. Then one of the horns of the uterus was cooled after a booster dose. Development of decidual tissue in the uterus was observed in these conditions. The initial signs of formation of this tissue was noted in 4 hours while completely formed decidual tissue was found in 3 days.

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METHODS

A NEW METHOD OF FORMING A FISTULA OF THE COMMON BILE DUCT IN DOGS

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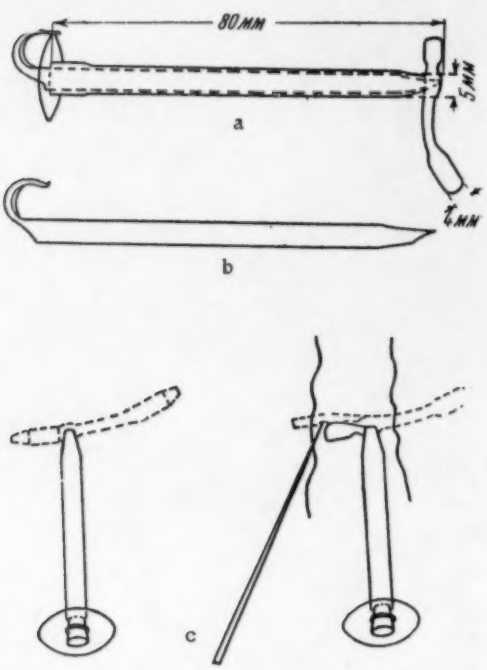
The study of bile formation and excretion is of both theoretical and practical importance. This problem has for many years attracted the attention of physiologists and clinicians.



Sketch of the fistula tube. a) Fistula tube with accessory tube inserted; b) accessory tube; c) diagram of the sequence of insertion of the fistula tube in the duct.

It must be pointed out that all the information obtained on bile formation and excretion is based on the results of the I. P. Pavlov school, on dogs with chronic fistulas of the common bile duct and the gall bladder [1, 2, 3].

In the course of work in our laboratory on problems of bile formation and excretion we became convinced that the existing methods of formation of a fistula of the common bile duct have many essential defects. This is also mentioned in several reports in the literature [1, 2, 3, 4, 5].

The worst defects of the existing methods of formation of a fistula of the common bile duct are as follows:

1) The animal loses the whole of its bile through the orifice of the fistula.

2) Bile flowing through the orifice of the fistula spreads over the abdominal wall, often causing considerable irritation of the skin and in some cases even leading to the formation of marked eczematous changes.

3) From 4-6 months after the production of a chronic fistula of the common bile duct the excretion of bile through the orifice of the fistula sometimes ceases, since the bile finds an alternative route into the duodenum.

These considerations impelled us to turn our attention to the development of a new method of formation of a fistula of the common bile duct.

We started from the assumption that the method must satisfy the following demands: at other times than during the experiment the bile must pass into the duodenum, and during the experiment it must be collected freely in a vessel.

EXPERIMENTAL METHOD

In carrying out these experiments a special fistula tube was made from copper plated with chromium or silver (it is best to use a silver tube). The fistula tube is T-shaped (seefigure). The horizontal part of the tube is 30 mm long. The diameter of the tube at its ends is 4 mm and the remainder is thicker which permits easier retention of the tube in the bile duct and also makes it possible to apply a ligature. The vertical part of the fistula tube is 70-80 mm long and 5-5.5 mm in diameter.

At the time of the experiment an additional hollow tube with obliquely cut ends is inserted in the fistula tube just described in order to close the outlet of bile into the intestine and to direct it externally.

On the external end of the fistula tube there is a round metal cap to prevent the tube being drawn into the abdominal cavity.

For the operation it is best to choose a dog with a wide thorax. Under general morphine and chloroform anesthesia an incision 8-10 cm long is made in the abdominal wall along the linea alba. The duodenum and pancreas are brought out through the incision. The common bile duct is identified. It is easily found by traction on the duodenum. When the duct has been found two ligatures are passed under it at a distance of 15 mm apart. A longitudinal incision 8 mm long is made in the duct between the ligatures (it is not advisable to dissect out the duct between the ligatures in order to avoid damage to the nerves and blood supply of the duct). By means of a hook the long horizontal limb of the fistula tube is inserted through the incision into the duct on the side of the liver as far as it will go (see figure, c). Next the opposite end of the incision is lifted up with the hook and the second limb of the fistula tube inserted, directing it towards the duodenum. The incision in the duct is then sutured around the thin part of the tube, and the two ligatures previously applied are tied lightly (they must not be tied tightly to avoid necrosis of the duct and interference with its blood supply).

After the fistula tube has been sutured the duct is covered with the free end of the omentum to improve the healing of the wound.

The incision in the abdominal wall is closed in layers. During this process the free end of the fistula tube is sutured into the anterior end of the incision. This concludes the operation.

EXPERIMENTAL RESULTS

Observations over a period of 13 months on animals undergoing operation by this method have shown that the dogs appear quite fit; no disorders of the digestive organs are observed. Outside the experiments the bile passes to the duodenum. During the experiment all the bile can be directed externally by inserting the accessory tube.

SUMMARY

Experiments were performed on dogs. This method gives an opportunity to collect bile into tubes during the experiment, at other times it enters the intestine, as in normal conditions. With this purpose a T-shaped tube is inserted into the common bile duct. Its horizontal part is introduced into the incision of the duct, while the vertical part comes out through the abdominal wall.

For suturing the tube an incision of the abdominal wall is made along the linea alba. The duodenum and the pancreas are exposed. 2 ligatures are placed under the bile duct, the distance between them being 15 mm. Then a longitudinal incision of the duct is made and the horizontal part of the fistula tube is inserted. The opening in the duct is sutured and covered by the omentum. The incision of the abdominal wall is also sutured with the exception a small opening through which the vertical part of the fistula tube comes out. In 7 to 10 days one may commence to collect and examine the bile.

This method has a number of advantages over the existing ones. Observations of dogs (8 months) operated by this method demonstrated that the process of digestion, as well as the processes of formation and secretion of bile is quite normal.

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STUDY OF PANCREATIC AND BILIARY SECRETION IN HORSES BY MEANS OF A CHRONIC FISTULA

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The Pavlov fistula method has been used successfully by numerous physiologists to study the physiology of digestion in various species of animals. It has made possible not only the discovery of distinctive features of the activity of the digestive glands in different animal species but also the proper organization of a rational diet.

Investigations on horses using the fistula method have revealed distinctive features of the secretion of saliva and bile in horses on different diets in a natural form or after technological processing or enrichment with various additives. The effect of the feeding program and of its content on the functional activity of these glands has been studied.

However, a wide gap in the investigation of the physiology of digestion in horses until recently has been the lack of data on the physiology of the pancreas. Whereas the physiology of this gland has been studied in other species of animal by means of the fistula method, in the horse on account of the difficult operative access there have been no reports of the successful formation of a fistula of the pancreatic duct. The only known papers in the literature are those of Colin [3] and of N. A. Popov and A. A. Kudriavtsev [1], who performed acute experiments on horses. However, in these papers the results given are only of the composition of the pancreatic juice of the horse.

The department of physiology of the Moscow Veterinary Academy, under the direction of N. F. Popov, has for many years been working on the problem of creating a permanent pancreatic fistula in the horse. In 1955 for the first time we performed the operation of formation of a fistula of the pancreas and the bile duct in a horse (see figure).

EXPERIMENTAL METHOD

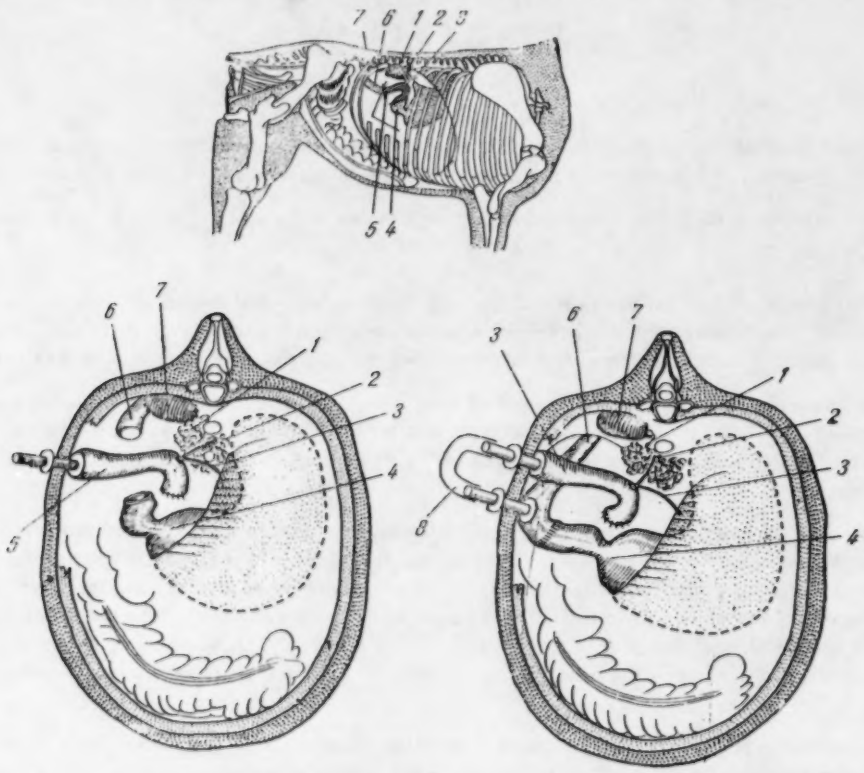
In operations on the pancreas in horses the main difficulty is in the complicated access, on account of the anatomical peculiarities of the disposition of the stomach, duodenum, pancreas and bile duct.

In the horse the stomach is situated mainly in the left hypochondrium at the level of the 13th to 15th ribs and only its pyloric part reaches into the right hypochondrium where it is situated at the level of the upper third of the 11th to 13th ribs. The stomach is well fixed by ligaments to other organs. The immobility of the stomach creates great difficulties for the experimenter. The access to the pyloric portion of the stomach is particularly complicated and is possible only through the right half of the thorax.

The duodenum is equally immobile and deeply situated. Beginning at the pylorus it forms an S-shaped flexure on the visceral surface of the liver and is directed superiorly and posteriorly to the right kidney. The pancreatic ducts open into the duodenum at a distance of 12-15 cm from the pylorus in the S-shaped flexure. They are usually two in number: one – the main duct, opening jointly with the bile duct, the other – the accessory duct, opening into the duodenum at the same level. Thus, because the main pancreatic duct takes a course in the same connective tissue sheath as the bile duct and opens jointly with it into the duodenum, the formation of a fistula of the pancreatic duct in isolation from the bile duct has not appeared to be possible.

Pancreatic juice could be obtained only by resection of the part of the duodenum into which the pancreatic and hepatic ducts open. The approach to the site of opening of the ducts in the usual lateral position of the horse is inconvenient on account of the impossibility of bringing this part of the duodenum up into the wound. For this reason the horse was fixed in the dorsoventral position.

The operative approach to the abdominal cavity was made through the right hemithorax by resection of the 13th to 16th ribs. The main incision passed along the bed of the 16th rib from the angle to the costochondral junction and then forwards at an angle horizontally to the 12th rib. In order to prevent a pneumothorax the diaphragmatic layer of the pleura was sutured to the parietal layer along the whole length of the incision.



Scheme of the operation for formation of a permanent fistula of the pancreatic duct and bile duct in the horse. 1) Pancreas; 2) pancreatic duct; 3) bile duct and liver; 4) pyloric portion of the stomach; 5) resected portion of the duodenum; 6) duodenum; 7) right kidney; 8) external anastomosis between the fistulas.

In order to resect the part of the duodenum with the outlets of the pancreatic and bile ducts, the pyloric part of the stomach and the first part of the duodenum and part of the duodenum from the S-shaped flexure were brought into the wound and fixed. Next the part of the duodenum in the S-shaped flexure, i.e., from a point 6-7 cm beyond the pylorus to a point immediately beyond the flexure, was resected. The cranial end of the resected portion of the duodenum was sutured, and into the caudal end was inserted a metal cannula which emerged from the upper end of the wound. The patency of the intestine was restored by the formation of an anastomosis. A second metal cannula was inserted into the part of the intestine distal to the anastomosis and brought out of the lower end of the wound.

In order to prevent a copious loss of pancreatic juice and bile (this leads not only to disturbance of the normal digestive processes but also to the rapid death of experimental animals) both cannulas were joined by means of an external anastomosis using a rubber tube. Thus, the juice and bile secreted during the experiment is collected in a flask while that secreted at other times passes into the intestine and there is no disturbance of the normal process of digestion. Pancreatic juice mixed with bile began to be excreted immediately after the operation through the cannula inserted into the intestine.

EXPERIMENTAL RESULTS

Experiments were carried out on 2 foals on which these operations had been undertaken in order to study the secretory activity of the pancreas and the secretion of bile. The experiments showed that the secretion of pancreatic juice and bile in the horse is continuous; there was no cessation of secretion even after starvation for 42 hours. Secretion of juice takes place rhythmically with intervals of 1-2 and sometimes 4-5 minutes.

The taking of food sharply stimulates the secretion of pancreatic juice and bile. In order to discover the mechanism of secretion of pancreatic juice and bile at times unrelated to the taking of food, we carried out experiments in which injections of 0.1 and 0.3% solutions of hydrochloric acid and also horse gastric juice, obtained from an isolated gastric pouch, were given into the duodenum. It was observed in these experiments that the hydrochloric acid solutions and the gastric juice cause a considerable increase in the spontaneous secretion of pancreatic juice and bile within 15 minutes of their injection. The volume of secretion depends on the concentration of the acid solution injected; the higher the concentration of the acid the greater the volume of secretion caused.

The Secretion of Pancreatic Juice and Bile in the Horse on Hay, Oats and Bran (the foal Orlik)

Time and conditions of experiment	Volume of jufce in ml	pH of juice	Digestion of starch in mm of a starch stick	Lipolytic power of the juice in units of 0.1N solution of alkali	Food
Experiment dated January 29, 1955					
Before feeding . . .	320	8.10	0,75	26	
After feeding:					
1st hour	477	7.84	1.0	24	1 kg of hay
2nd »	501	7.47	0.5	22	
3rd »	523	7.33	1.0	36	
4th »	498	7.32	1.5	44	
Experiment dated January 31, 1955					
Before feeding	284	7.32	4.0	26	
After feeding:					
1st hour	519	7.77	3.0	22	
2nd »	557	7.80	3.5	24	1 kg of oats
3rd »	569	7.79	3.0	26	
4th »	546	7.78	3.0	30	
Experiment dated February 5, 1955					
Before feeding	292	7.52	1.2	20	
After feeding					
1st hour	559	7.37	1.5	26	
2nd »	585	7.01	1.5	26	1 kg of wheat bran
3rd »	637	6.64	1.6	28	
4th »	576	7.35	2,0	30	

The experiments carried out confirm the findings obtained in other animals: gastric juice, and in particular its acid are the connecting links between the secretion of gastric and pancreatic juice. The experiments also demonstrate the effect which the activity of the glands of the stomach can exert on the secretion of pancreatic juice in the horse.

In a study of the secretion of pancreatic juice and bile in response to different forms of food, we established that the ingestion of different foods causes an unequal secretory reaction, both as regards the volume of juice secreted and its enzymic content (see table).

The highest secretion of juice is observed during feeding with wheat bran. Ingestion of bran causes within one hour a significant increase in the volume of juice which is more than twice the initial level. The maximum rise in secretion takes place in the 3rd hour and in the 4th hour the secretion falls slightly. The enzymic content of the juice rises about the 4th hour of secretion.

During feeding with oats an increase in the secretion of juice is also observed within an hour of taking food and its maximum rise is in the 3rd hour. However, during feeding with oats the volume of juice secreted is smaller than during feeding with bran. The enzymic activity of the juice in respect to its lipolytic enzyme is increased about the 4th hour and in respect to its amylolytic enzyme stays at almost the same level during the four-hour period.

The curve of secretion when hay is eaten is the same as with oats or bran. However, the volume of juice is significantly smaller than when the horse is fed on oats or bran. The enzymic activity of the juice is the same as when fed on oats, except that its starch-digesting power is slightly lower.

Salted oats causes an intensive reaction, especially within an hour of feeding. Feeding with cooked oats was not accompanied by any increase in the spontaneous secretion of pancreatic juice and bile.

The experiments described show that feeding with various types of fodder causes a different reaction on the part of the pancreas and the secretion of bile in the horse.

Consequently the creation of a permanent fistula of the pancreas and bile duct in the horse permits the study of the distinctive features of digestion in that animal as determined by the working of all the principal digestive glands – salivary, gastric and pancreatic. In this way physiological data may be obtained which may serve as a basis for the correct organization of the feeding of healthy and sick horses.

SUMMARY

A method of placing a fistula on the duct of the pancreas and bile duct of a horse was developed.

The effect of various food contents and regimen of feeding on the gastric and bile secretion was studied by means of this method.

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A CHRONIC FISTULA OF THE INTESTINAL LYMPHATIC DUCT OF THE CAT

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The principal investigations of the absorption of foodstuffs into the lymphatic system from the gastrointestinal tract were carried out either by the use of a fistula of the thoracic duct or during acute experiments on anesthetized animals. In spite of the obvious defects of these methods they are still used by experimenters today.

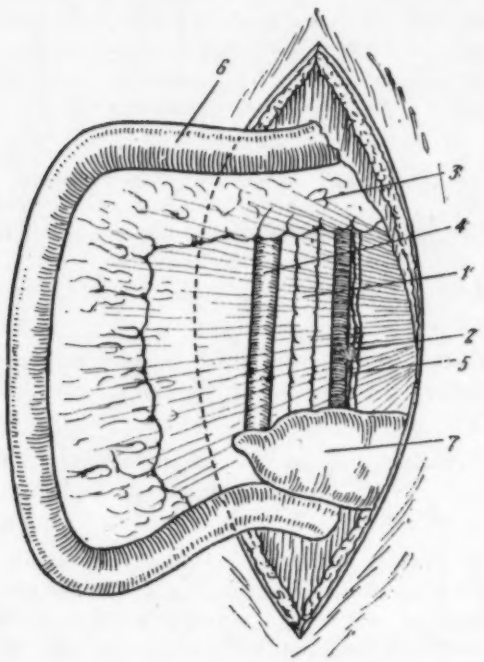

Fig. 1. Topography of the mesenteric lymphatics of the cat. 1) Main lymphatic duct of the small intestine; 2) cranial mesenteric artery; 3) pancreas; 4) cranial mesenteric vein; 5) lymphatic duct of the large intestine; 6) duodenum; 7) cecum.

In order to find out the possibility of developing this present method, a preliminary study was made of the anatomy of the lymphatic vessels of the gastrointestinal tract of various laboratory animals. In dogs, in which digestive processes have been studied in the greatest detail, the distribution of lymphatic vessels in the mesentery is variable and they constantly anastomose with each other by means of slender vessels which cannot be seen without the use of special methods. The most suitable experimental animal for these purposes is the cat.

There are usually three efferent lymphatic vessels from the mesenteric glands in the cat: 1) a vessel from the ganglia receiving lymph from the large intestine which lies to the left of the cranial mesenteric artery; 2) the main intestinal lymphatic duct, which is situated between the cranial mesenteric vein and the cranial mesenteric artery; 3) a third, slender lymphatic vessel from the duodenal gland, to the right of and dorsal to the cranial mesenteric artery (Fig. 1). All these vessels become confluent close to their point of entry into the cisterna chyli.

In the living animal the main intestinal lymphatic duct is easily visible after a milk feed 1-2 hours before the operation.

We used the principle of inserting the lymphatic vessel into a tube to develop a simple method of forming a permanent fistula of the intestinal lymphatic duct. As a fistula tube we used paraffin-coated polyvinyl tubes 3-4 cm in length and with an internal diameter of 1.5-2 mm. At one end of the tube 4 longitudinal

incisions were made, 4 mm in length and equidistant from each other. Immediately after sterilization in paraffin the strips formed as a result of the incisions in the tube are bent at a right angle. As suture material we used thread made from polyamide resin with a cross section of 0.15-0.3 mm.

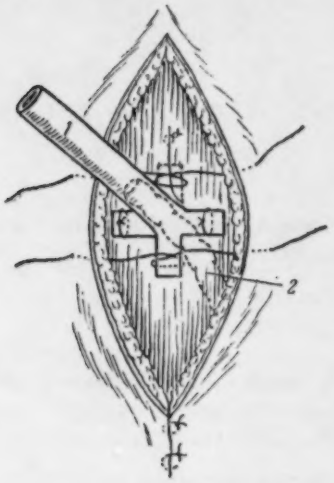

Fig. 2. Fixation of the fistula tube. 1) Fistula tube; 2) lymphatic duct.

The operations were carried out under intratracheal ether anesthesia. A longitudinal skin incision, about 5 cm long, was made from above downward , starting at the level of a line joining the subcostal arches and situated 3 cm from the midline on the right. A longitudinal incision was made in the linea alba of the abdomen, about 4 cm long, at the same level.

The proximal section of the large intestine and the duodenum with their mesenteries were brought out of the operation wound. The main lymphatic duct was identified in the mesentery between the cranial mesenteric vein and the cranial mesenteric artery and ligated 1.5-2 cm distal to the lymphatic gland. The portion of the lymphatic vessel situated between the ligature and the gland was swollen from the lymph entering it while the remaining portion of the vessel was collapsed. The collapsed portion of the vessel was completely divided between ligatures. The distended end of the lymphatic vessel was mobilized by blunt dissection for a distance of 1 cm. In doing this we strove to spare the blood vessels supplying the wall of the lymphatic vessel. Next the lymphatic vessel was incised for $^2/_3$ of its diameter near the ligature and by the aid of this ligature the vessel was drawn into the tube – the tube as it were was clothed over the lymphatic vessel.

Two of the strips of tube ("paws"), bent at a right angle, were fixed with sutures to the peritoneum of the mesentery. If there were a space between the base of the tube and the peritoneum a purse-string suture was inserted. The ligatures, together with the partly divided vessel were withdrawn by careful traction. As a result of this the lymphatic vessel within the tube remained free. The base of the tube, together with the mesentery were fixed by means of the other two "paws" to the abdominal wall when this was sutured (Fig. 2) and the free end of the tube exteriorized through a stab incision made in the skin in the midline.

After the operation the cats were kept at liberty in a warm and commodious room in which the experiments were conducted. The cats were familiarized with convenient soft bedding on which they lay throughout the experiment. The lymph was collected in a vessel fixed under the animal.

Excretion of lymph through the fistula continued for 6-7 days. The cessation of excretion is explained by the development of a connective tissue scar at the base of the tube, and drainage of lymph from the intestine takes place subsequently along developing collaterals.

We are in agreement with the view of Freeman [6] and D. A. Zhdanov [1] that all the lymph flowing from the organ cannot be judged from the amount which flows along this particular vessel on account of the presence of numerous anastomoses. These anastomoses, like a proportion of the efferent lymphatics, cannot be demonstrated in the living animal [2]. Hence we consider that when investigating the intestinal lymph special attention must be paid to its qualitative indices.

The volume and composition of the lymph excreted through the fistula was dependent on the diet. The refractometric index varied from 1.3360 after intake of water to 1.3565 in cats fed on milk. The volume of intestinal lymph excreted during half an hour in starving cats was about 0.1 ml and after a milk feed it reached 20-24 ml.

An experimental study was made of the refractometric index after water intake.

The experiments were carried out 2-3 days after operation, on animals deprived of food or water for 18-24 hours before the experiment. For the duration of one hour the refractometric index and the volume of lymph excreted were determined. Next, 100 ml of water at room temperature was injected into the stomach through a tube.

The refractometric index of the intestinal lymph after an intake of water constantly showed an extremely sharp change, falling from 1.3450-1.3440 to 1.3360 (Fig. 3).

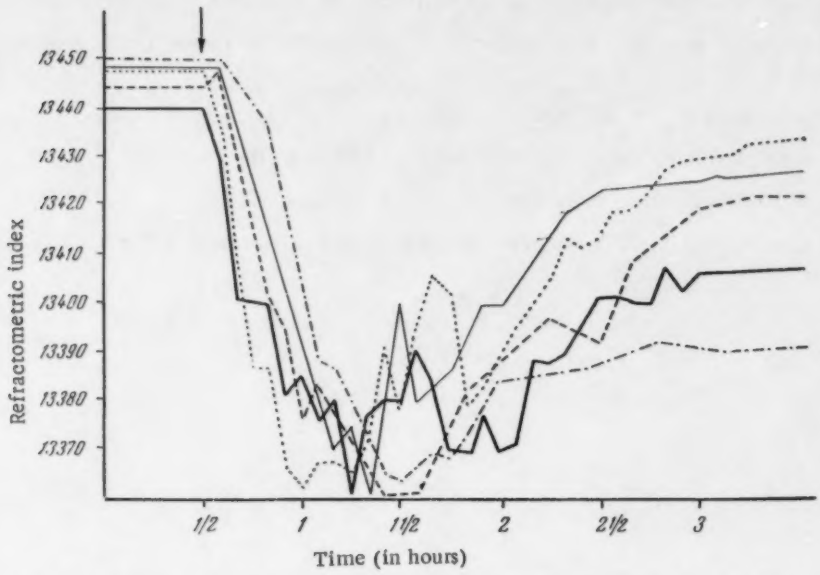

Fig. 3. Changes in the refractometric index of the intestinal lymph after injection of 100 ml of water through a gastric tube. Results of 5 experiments. The arrow indicates the moment of injection of the water.

We did not consider it possible to translate the refractometric index into protein values since this index is determined not only by refraction of protein but acts at the same time as an index of the total dry residue of the lymph.

The volume of lymph was increased after intake of water from 0.1 ml per ½ hour during the control period to 2.4-3 ml in 5 minutes, half an hour after giving the water, and it returned to its initial level after 2 hours.

Denial of the importance of the lymphatic system in the absorption of water from the gastrointestinal tract evidently needs re-examination, and the result obtained by Bollman and his co-workers [3, 4, 5, 7] must be given an alternative interpretation.

In confirmatory experiments on anesthetized animals, like other workers we were unable to observe any essential changes in the refractometric index and the volume of lymph. The same results were obtained in experiments on cats with a fistula, but placed in a special cage for fixation.

Anesthesia and fixation of the animal evokes significant functional changes which do not allow study of the normal physiological features of absorption of food products into the lymphatic system.

The method described, as our results show, can be used for the study of absorption of all the food products into the lymphatic system from the gastrointestinal tract.

SUMMARY

A new method of obtaining a chronic fistula of intestinal lymphatic duct in cats, functioning for 6-7 days is described. The lymph from the fistula was examined in conditions of a chronic experiment. It was revealed that the refractometric index after forcing fluid is decreased from 1.3450 – 1.3440 to 1.3360, while the quantity of the lymph is increased from 0.1 for half an hour to 3 ml within 5 minutes. The same experiments were carried out on anesthetized cats – no significant changes of refractometric index or of the quantity of the lymph from the fistula could be noted after forcing fluids.

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* In Russian.

A GRAPHIC METHOD OF RECORDING THE ACT OF YAWNING AND OTHER FORMS OF MOVEMENT OF THE MOUTH

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(Received July 20, 1957. Presented by Active Member of the AMN SSSR V. V. Parin)

During the investigation of the motor components of unconditioned and conditioned reflexes a good deal of attention has been paid to the study of movements of the mouth [1, 2, 3].

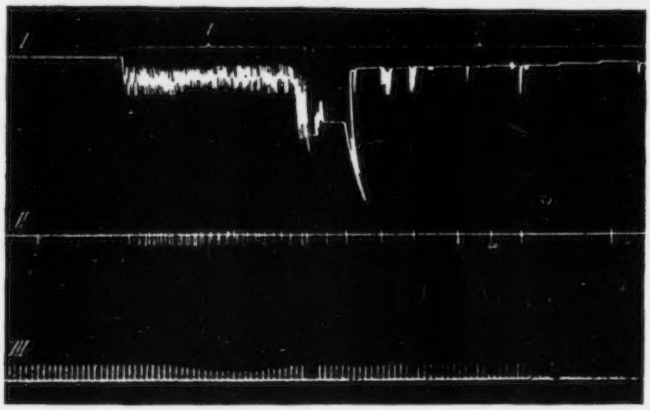

Fig. 1. I) Tracing of movements of the mouth: 1) during eating, 2) licking; II) tracing of secretion of saliva; III) time marker (2 seconds).

The methods suggested for recording the movements of the mouth in dogs are based on the principle of air transmission: the mouth of the animal is surrounded by a rubber band, and when yawning movements occur and the mouth is opened this transmits pressure to a rubber balloon attached under the lower jaw or to the dorsum of the nose of the animal.

However, when the amplitude of the movements is great, for example, during the act of yawning or in some forms of defensive reflexes, the pneumatic method is unsound because in such cases the balloon will always experience the maximum pressure and the tracing will show the mouth movements disproportionately.

A silk thread, many times longer than the circumference of the wide-open mouth of the animal, is glued at its center to the skin of the lower jaw of the animal. Both ends of the thread, passed round the muzzle of the dog on each side, are taken through the limbs of a light glass Y-tube into a composite glass tube, fixed at one end to a cork disc which is glued to the skin of the dorsum of the nose of the animal, and at the other end to the cross-beam of the stand. The composite glass tube is formed of sections of drawn glass tubing joined together with rubber connecting tubes. The length of the tube is sufficient to allow free change of position of the animal on the apparatus. The ends of the loop of thread, after passing through the composite glass tube, are connected

to the recording lever and with each increase in the circumference of the loop the pen of the recording lever is deflected downwards. The return movement of the lever, which is counterpoised, occurs when the diameter of the loop is reduced as the animal closes its mouth. The composite glass tube system effectively damps any general movements.

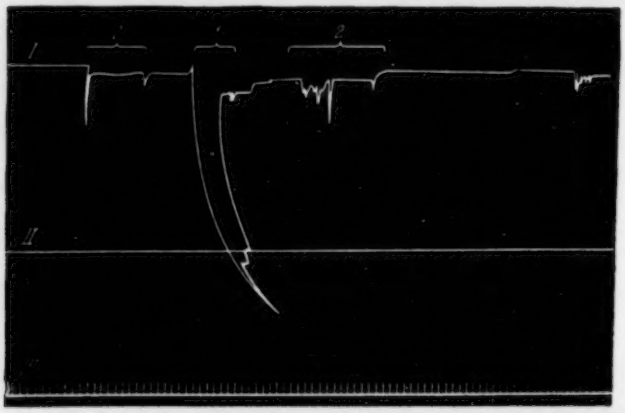

Fig. 2. I) Tracing of movements of the mouth: 1) yawning, 2) licking; II) tracing of secretion of saliva; III) time marker (2 seconds).

Since the length of thread inside the glass tube at any given moment cannot change appreciably, the only part of the silk thread which can affect the position of the recording arm is the loop around the animal's mouth. Since this loop is freely extensible to any degree to which the animal may open its mouth, the recording will reflect completely the character of the movements of the mouth.

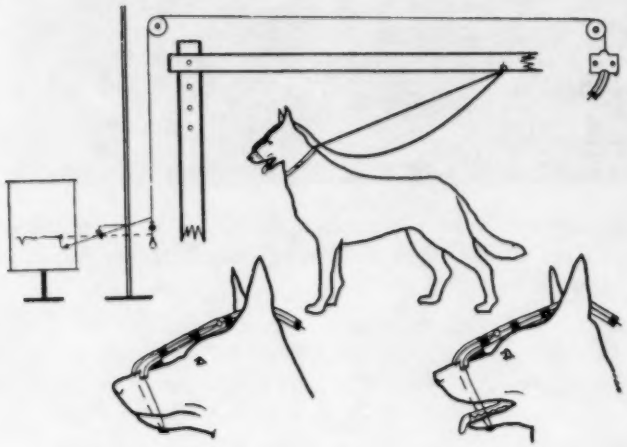

Fig. 3. Diagram of recording of movements of the mouth.

The method suggested may be used in tracing the mouth movements with amplitudes from the very slightest to the maximum.

The outline of the recording and the form of the tracing are shown in Figs. 1, 2 and 3.

SUMMARY

Mechanical transmission by way of a silk thread contained in a glass tube is proposed for registration of mouth movements. Registration of all forms of mouth movements is possible.

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RECONSTRUCTION OF THE EKP-4M ELECTROCARDIOGRAPH AS A TWO-CHANNEL APPARATUS

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(Received August 1, 1957. Presented by Active Member of the AMN SSSR V. V. Parin)

In many cases during clinical and physiological research it is desirable to make a simultaneous record of several phenomena.

In recording a ballistocardiogram, for example, it is essential to establish the time correlations of the waves of the ballistocardiogram and the electrocardiogram. In the absence of a multi-channel recording apparatus it is possible to use for this purpose an EKP-4m apparatus, taking one of the leads of the electrocardiogram to the ballistocardiogram. The curve obtained in this way is of some diagnostic value but there is inevitably some distortion of both the shape and amplitude of the ballistocardiographic waves. For this reason we decided to redesign the EKP-4m electrocardiograph, incorporating a second channel in it, completely identical with the first (a set taken from a second EKP-4m apparatus).

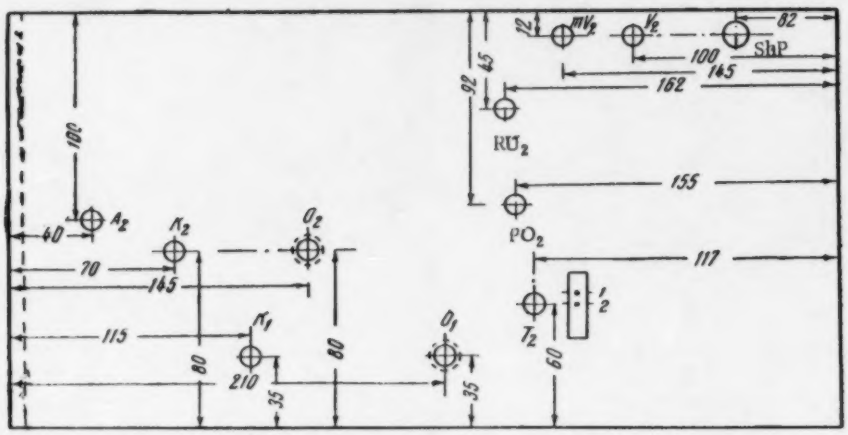

Fig. 1. Plan of the control panel of the supplementary channel (measurements in millimeters) to the EKP-4m apparatus. A_2 – Supplementary switch for BAS_2; K_2 – corrector of the supplementary channel; O_2 – illuminating light of the supplementary channel; T_2 – tumbler switch for the supplementary channel; PO_2 – switch for the leads from the supplementary channel; RU_2 – amplification regulator of the supplementary channel; mV_2 – push-button (millivoltmeter) of the supplementary channel; Shp_2 – leads to the patient from the supplementary channel; K_1 – corrector of the main channel; O_1 – illuminating light of the main channel; 1) center of the beam of the main channel; 2) center of the beam of the supplementary channel.

As our experiment showed, the reconstruction of the apparatus is not very complicated and moreover, it permits a perfectly steady simultaneous recording to be made of the ballistocardiogram and the electrocardiogram.

The apparatus was modified in the following sequence: 1) all the knobs are taken from the control panel; 2) the ebonite (facing) panel of the apparatus is removed; 3) the partition which separates the filament battery is moved 14 mm to the left, which frees space for a second galvanometer (the filament battery [2 NKN-10] like the anode batteries can be controlled by an outside switch); 4) the supplementary galvanometer with its corrector is mounted 70 mm from the left border of the upper panel and 80 mm from the lower border; 5) the existing galvanometer with its corrector is moved 25 mm downward ; 6) in line with the supplementary galvanometer and at a distance of 145 mm from the left border of the panel is mounted the illuminating light of the supplementary galvanometer; 7) the illuminating light of the existing galvanometer is moved 25 mm downward ; 8) the upper front portion of the middle partition of the apparatus is removed; 9) the charging system is transferred to the partition mentioned in item 3, from which a window measuring 40 × 25 mm is cut out in order to connect the terminals of the flex during charging of the filament battery; 10) the amplifier of the supplementary channel, on shock-absorbing springs, is mounted in the place formerly occupied by the selenium column; 11) at a distance of 92 mm from the right upper border of the panel and 144 mm from the right side is mounted the supplementary switch for the leads, made from a type PD-4-6 radio switch; 12) above the supplementary switch for the leads is mounted the supplementary amplification regulator; 13) behind the amplification regulator are placed 2 push-buttons: one for the voltage calibrator (MV), the other for the damper; 14) to the right of the push buttons a hole is drilled for

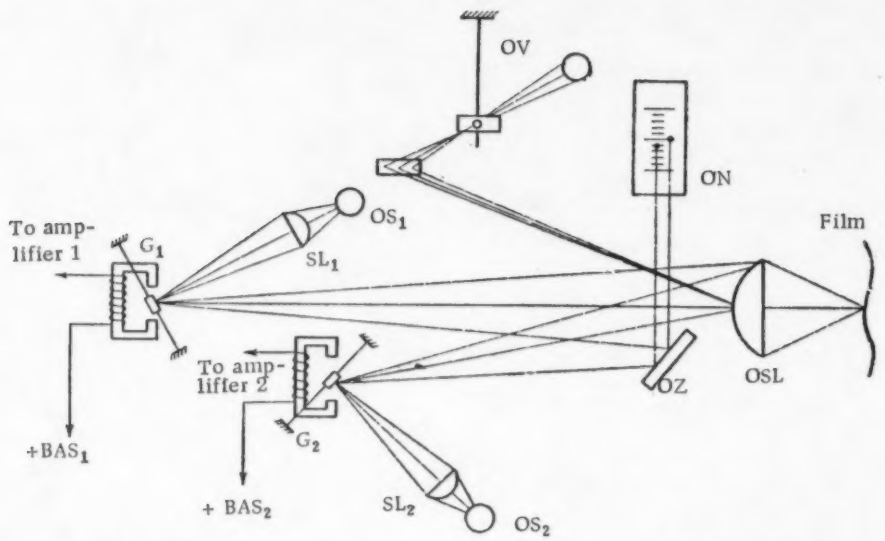

Fig. 2. Optic system of the reconstructed EKP-4m apparatus. G_1 – Newly installed galvanometer; $OS_1 - SL_1$ – newly installed illuminating light; G_2 – existing galvanometer; $OS_2 - SL_2$ – existing illuminating light; OZ – reflecting mirror; OSL – existing common collecting lens; OV – existing time marker; ON – existing visual control window.

coupling the supplementary triple lead from the patient to the circuit; 15) in the place formerly occupied by the transformer is placed the voltage divider of the calibrator (MV); 16) below the switch A, at a distance of 22 mm is mounted the switch for the second anode battery (common negative; the second battery is added to prevent harmful electrical connections between the 2 amplifiers through a common source of power); 17) next to the "On" tumbler switch is mounted a second tumbler switch; 18) the appropriate connections are made between the components of the circuit of the supplementary channel; 19) holes are drilled in the ebonite facing panel of the apparatus in suitable places for accomodating the axes of the supplementary equipment (Fig. 1) and the necessary inscriptions or signs are made; in view of the fact that after the reconstruction the galvanometers are "dispersed" and a slight angle is formed in relation to the collecting lenses, marks are placed on the window for visual observation corresponding to the center of the beam on the film; 20) the ebonite panel is replaced.

The optic system of the reconstructed apparatus is shown in Fig. 2.

The apparatus is simple in use. The patient's leads from the main channel PR and LR are connected to the ballistocardiographic output and those of the supplementary channel to electrodes fixed to the patient, as usual. Both tumbler switches are switched on and the lead switches are in the O position. By means of the correctors both beams ("crests") are fixed on the visual observation window so that the distance between them is sufficient to enable the curves to be distinguished and the required degree of amplification is established. The switch for the leads of the main channel, through which the recording of the ballistocardiogram is effected, are set in position 1. The switch for the leads of the supplementary channel are set in the required position and then the tape-winding mechanism is set in motion. If it be proposed to record the electrocardiogram only, then only the tumbler switch of the main channel is switched on and the electrocardiogram is recorded in the usual way.

SUMMARY

An apparatus for simultaneous registration of ballistocardiogram and electrocardiogram is recommended. A second channel which is identical to the first one is mounted into the EKP-4 m electrocardiograph.

A NEW DEVICE FOR EXCLUSION OF THE CIRCULATION OF THE INTERNAL ORGANS IN CHRONIC EXPERIMENTS

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The development of methods of temporary exclusion of the blood supply to internal organs and of various vascular regions in chronic experimental conditions is of great interest to the study of physiology, pharmacology and biochemistry in the intact animal. In contrast to the acute experiment, when the circulation can easily be interrupted by clamping the vessels operatively, using the usual hemostats, the temporary exclusion of the circulation in chronic experiments demands special devices.

In order to produce experimental hypertension Goldblatt and his co-workers [8] used a special clamp by means of which it was possible to achieve prolonged and constant constriction of the renal vessels in chronic experiments on dogs (Fig. 1, 1). This method has been developed further by many workers, but the apparatus suggested was suitable only for constant constriction of the lumen of vessels in chronic experiments.

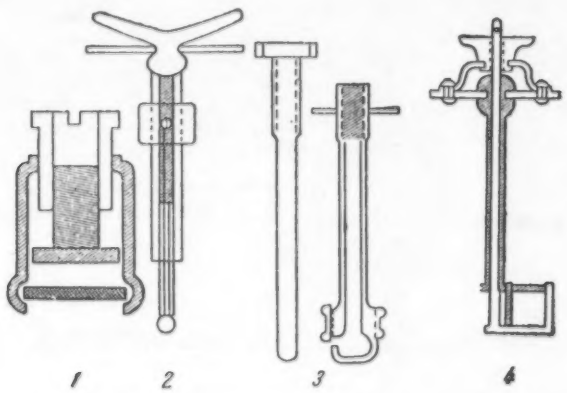

Fig. 1. Devices for excluding the circulation of internal organs. 1) Clamp described by Goldblatt and co-workers; 2) the loop-constrictor used by I. Smirnov; 3) the cannula devised by I. S. Kanfor; 4) vascular clamp (first variant).

I. Smirnov [3] used a special device – the loop-constrictor – for the temporary occlusion of vessels in the rabbit in acute experiments. This consists of a tubular body in the interior thread of which revolves a rod connected to a string; tightening the string constricts the vessel. The apparatus is equipped with a scale which permits graded constriction of the lumen of the vessel to be carried out.

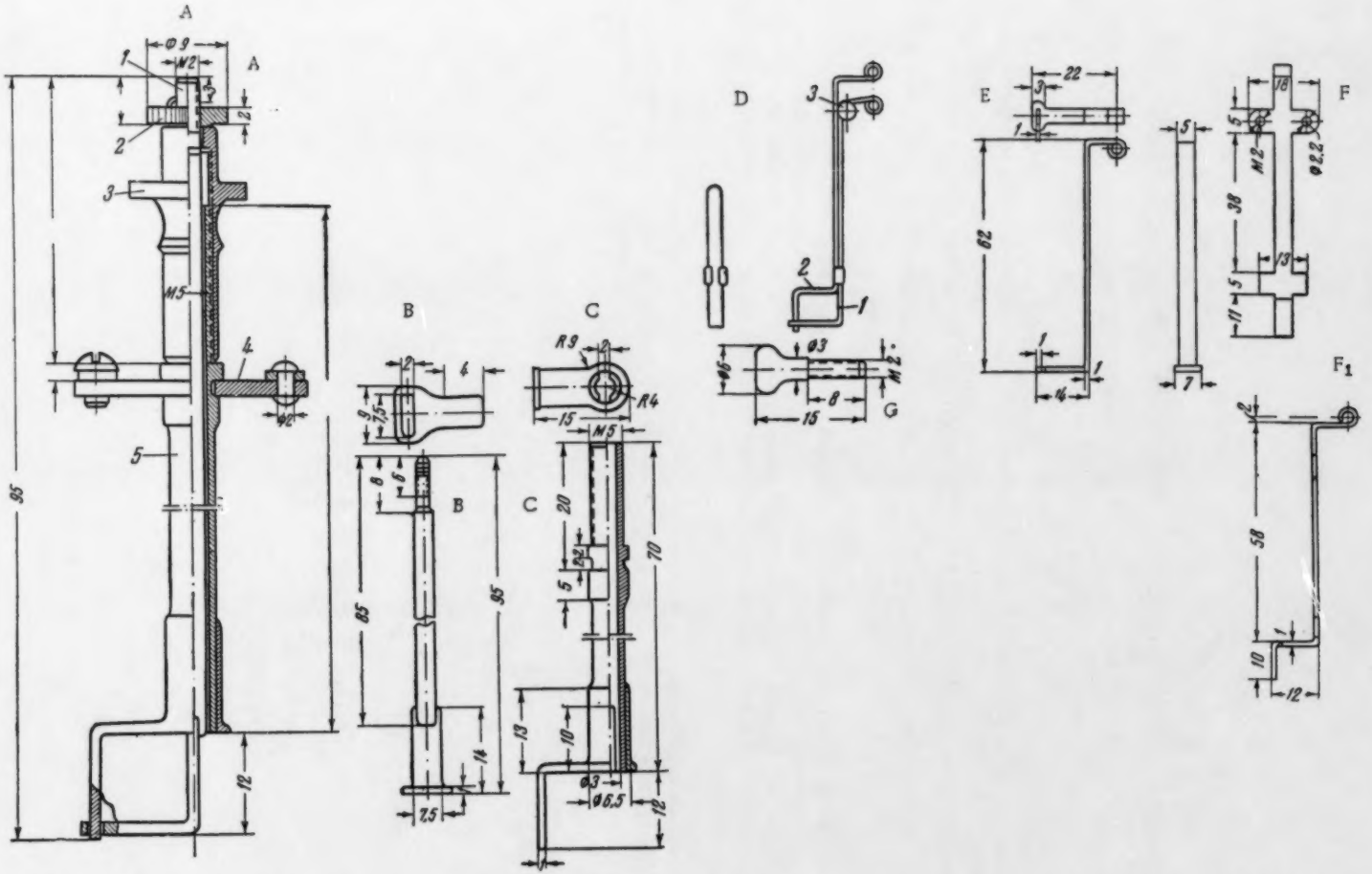

Fig. 2. The vascular clamps SZ-2 and SZ-3. A) The SZ-2 apparatus in assembled form; B) detail 1 of the apparatus SZ-2; C) detail 5 of the apparatus SZ-2; D) the SZ-3 apparatus in assembled form; E) detail 1 of the apparatus SZ-3; F) detail 2 of the apparatus SZ-3; G) detail 3 (screw) of the apparatus SZ-3.

I. S. Kanfor [1] devised a metal cannula which, like the previous author, he used in acute experiments [2]. This cannula consists of a tubular body in the interior thread of which revolves a rod which compresses the vessel against the base of the cannula. Thus, the vessel is compressed in the cannula between two flat surfaces – the immovable bed of the apparatus designed to take the vessels and the revolving butt end of the bolt; hence conditions are created for twisting and severe trauma to the wall of the vessel during its constriction. This can easily be proved by compressing an ordinary rubber tube between the revolving and immovable surfaces. These technical defects combined with the great weight of the cannula restrict its use to acute experiments only [2]. In this respect the cannula has no advantage over that previously suggested by I. Smirnov; there has never yet appeared any report of its successful use in a chronic experiment.

For temporary occlusion of the circulation of the gastrointestinal tract in chronic experiments we introduced in 1952 a new type of apparatus – a vascular clamp, differing in its construction from all the devices enumerated.

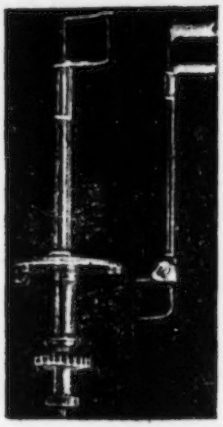

Fig. 3. Vascular clamps (SZ-3 apparatus without handles).

In this clamp which we developed the vessels are included in the lumen of a rectangle and are compressed by the approximation of parallel plates lined with rubber. Thus in the construction of its working parts the vascular clamp is closer to that described by Goldblatt than to Kanfor's cannula (see Fig. 1). By means of the vascular clamp it was possible for the first time in chronic experiments to carry out temporary occlusion of the circulation of almost the whole of the gastrointestinal tract in dogs [4-7]; the possibility of using this method on a wider scale was thus shown.

However, the vascular clamp was not faultless and called for further improvement. With this in view we constructed two new clamps SZ-2 and SZ-3 which are described below.

The newer models have a number of advantages over the first apparatus: simpler construction, lighter weight and safer constriction of the vessels.

The apparatus SZ-2 consists of 5 components (Fig. 2, A-C and Fig. 3). Detail 1 is ⌜ -shaped; at the end of its flat plate a slot is made to accommodate the vertical plate of detail 5, and at the opposite end there is a thread for the fixing nut 2 and an opening for the splint. On the internal surface of the cylindrical nut 3 there is a thread for engaging with the detail 5. A separating disc 4 is inserted in a cut-out area of the detail 5; it is designed for fixing the apparatus to the surface of the body and for engaging with the cylindrical nut. Inside the detail 5 a hole is drilled for the rod and directing slot for the plate of detail 1, while on the outer part there is a thread for engaging with the cylindrical nut and a cut-out area for fixing the separating disc.

During application to a vessel the rod of detail 1 is inserted in the hole in detail 5 and the vessel is inserted between the ⌜ -shaped plates of these details. Next the tubular part is brought out to the surface of the body through a trocar puncture. The cylindrical nut 3 and the fixing nut 2 are screwed on the thread of detail 5. In the hole in detail 1 is inserted the wire splint to prevent the nut 2 from unscrewing and releasing the clamp. Next the separating disc is inserted in the hollow in detail 5 and fixed by screwing tight the fixing screw of the disc. Compression of the vessel is effected by tightening the cylindrical nut 3 as far as possible (or until the required degree of constriction of the vessel has been attained) in an anti-clockwise direction. In order to restore the original situation the cylindrical nut is turned clockwise as far as possible.

The SZ-3 apparatus is of "periscopic type" and is very simple in construction and easily made from ordinary stainless steel plate. It consists of 3 components (Fig. 2, D-G). Its two flat plates, connected to each other, form a rectangle in which is inserted the vessel to be constricted. For this purpose the screw 3 is unscrewed, the detail 1 is displaced downwards (see 2 and 3) and the vessel is inserted between the ⌜ -shaped parts of details 1 and 2, forming the rectangle; these components are fixed by the screw 3 which prevents the clamp from opening and the vessel from slipping out. In preparing the apparatus for use, the plates which compress the vessel are coated with rubber. In order to minimize trauma, the long part of the apparatus may also be enclosed in a rubber tube. The

end of the clamp is brought out onto the surface of the body through an incision. When necessary the clamp may be fixed by a separating disc similar to that in the SZ-2 apparatus.

The vessel is compressed by a clamp of the Kocher type, for which purpose its ends are inserted into the handles of the details 1 and 2 (see Fig. 2, D). Restoration of the original position is achieved by separating the blades of the Kocher forceps which are then removed from the handles of the clamp.

The method of work with the SZ-2 and SZ-3 apparatus is similar to that described by us previously for the SZ-1 apparatus [4, 6]. We tested the two newer models of the vascular clamp in experimental occlusion of the circulation of internal organs in 2 dogs.

The vascular clamps described may be used for occlusion of the aorta and of its immediate branches. The measurements shown on the diagrams of the clamps are calculated for constriction of the abdominal aorta or of its immediate branches in large dogs. Depending on the dimensions of the vessels and on the regional topographical anatomical relationships the dimensions of the apparatus or of its component part may be modified.

We consider that the development and perfection of metal clamps does not finally solve the problem of creating a satisfactory device and method of occlusion of the circulation of internal organs in chronic experiments. The pneumatic method, in the development of which we are engaged at the present time, presumably offers greater promise.

SUMMARY

2 new variants of vascular clamps for temporary exclusion of the blood supply to internal organs in chronic experiments are described. The technique of application of vascular clamps was described formerly (the Bulletin of Experimental Biology and Medicine, No. I, 1955, pages 72-74).

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* Original Russian pagination. See C. B. Translation.
** In Russian.

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED

[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiia	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiia	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Embriol.	Arkhiv Anatomii Gistologii i Embriologii	Archives of Anatomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiia	Biokhimiia	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiia Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Biull. Eksptl. Biol. i Med.	Biulleten Eksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Biull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Biulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Eksptl. Khirurg.	Eksperimentalnaia Khirurgiia	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiia i Toksikologiia	Pharmacology and Toxicology
Farmatsiia	Farmatsiia	Pharmacy
Fiziol. Rastenii	Fiziologiia Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenova Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariia	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiia Akademii Nauk SSSR, Seriia Biologicheskaia	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiia Tikhookeanskogo N. I. Instituta Rybnogo Khoziaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiia	Khirurgiia	Surgery
Klin. Med.	Klinicheskaia Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaia Parazitologiia i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaia Radiologiia	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiia	Mikrobiologiia	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhiat. i Psikhogig.	Nevropatologiia, Psikhiatriia i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediia, Travmatologiia i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriia	Pediatriia	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Endokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniia Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaia Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journals
Stomatologiia	Stomatologiia	Stomatology
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriia	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics

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Abbreviation	Journal	Translation
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiia	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiia	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiia, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoiuz. Gidrobiol. Obshchestva	Trudy Vsesoiuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoiuz. Inst. Eksptl. Med.	Trudy Vsesoiuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiia	Urologiia	Urology
Uspekhi Biokhimiia	Uspekhi Biokhimiia	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiia	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriia Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universiteta, Seriia Biologii i Pochvovedeniia	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiia	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariia	Veterinariia	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniia	Voprosy Pitaniia	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaia Laboratoriia	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiia	Journal of General Biology
Zhur. Vysshei Nerv. Deiatel.	Zhurnal Vysshei Nervnoi Deiatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

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